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Development of Salt Tolerance in Sugarcane Variety Isd 16 through Tissue Culture Technique

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ABSTRACT

To study the regeneration potentiality of sugarcane variety Isd16 and development of tolerance against critical limit of NaCl concentrations the unexpanded spindle leaf sheaths were used as explants. For the development of salt tolerance in sugarcane variety Isd 16 MS medium stressed with various NaCl levels (50, 100, 150 and 200 mM) along with a check. The explants were aseptically cultured on MS medium supplemented with 2, 4-D (3.0 mgl-1) and coconut water (10%) for callus induction. Callus growth was vigorous on medium (control) as well as at lower concentration of NaCl. The frequency of callus induction decreased with the increased concentration of salt. Callus colour was found to be reddish where NaCl salt was added in the medium. For shoot regeneration and multiplication, a medium containing 2.0 mgl-1 BAP + 1.0 mgl-1 Kn was used. Regenerated shoots were rooted on MS medium supplemented with 5.0 mgl-1 NAA. Shoot formation and multiplication and root producing capacity decreased with the increase of salt concentration in MS medium. Most of the calluses became black and finally died within 35 d of incubation on medium supplemented with 200 mM of NaCl salt. Successful regeneration of plantlets with sufficient roots on medium supplemented with 50 and 100 mM NaCl salt indicated the moderate salt tolerance ability in variety sugarcane Isd 16.

Key words: Sugarcane, variety Isd 16, tissue culture, salt tolerance.

INTRODUCTION

Soil salinity is one of the most important environmental stresses limiting productivity of crops around the world. In Bangladesh sugarcane is an important cash-cum-industrial crop having poor yield (about 50 tha-1). About 0.833 million hectares of available lands of southern districts of Bangladesh are affected by varying degrees of soil salinity. Sugarcane cultivation in saline areas received no attention in the past. But due to increased demand of sugar and gur for local consumption it has become imperative to explore potentials of these lands where sugarcane is being cultivated years together without adopting modern technologies. Sugarcane varieties those are cultivated in saline prone areas do not have proven capabilities to grow under extreme stress conditions. Therefore, it is essential to develop improved varieties, suitable for salinity stress prone areas.

Since traditional plant breeding methods have been slow to yield substantial improvement in stress tolerance especially salt tolerance, the alternative approach of utilizing potential plant cells for the development salt tolerance in sugarcane has received increased research attention in a recent past. The tissue culture technique provides an important method for enhancing salt tolerance through exploiting genetic variability (Staverek and Rains, 1984; Chandler and Thorpe, 1986). In vitro selection for salt tolerance is commonly achieved through adaptation by compartment of excess salt in the vacuoles and survives by adjusting the osmotic pressure in hostile environment. Increased NaCl salt concentration in medium causes specific modification of plant morphology i.e., induced habituation where high level of NaCl act as selective and mutagenic agent (Tanvir *et al.*, 2001). It is claimed that if cell can adapt to a high salinity it could give rise to a plant which would similarly be stress tolerant (Gollek, 1973). Considering above facts the present investigation was undertaken to develop salt tolerance in sugarcane variety Isd 16 through tissue culture technique.

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MATERIALS AND METHODS

The experiment was carried at Biotechnology Laboratory of the Bangladesh Sugarcane Research Institute (BSRI). Sugarcane variety Isd 16 was used as explants source. Tops (20-30 cm long) of 3-5 months old field grown sugarcane plants of said variety were collected. The outer leaf sheaths were removed and chapped into segments of about 5 cm long. Prepared shoots were taken in a beaker and treated with 1% savlon for 5-6 min with constant shaking, and washed thoroughly with distilled water for 2-3 min. The explants were transferred in autoclaved conical flask (500 ml) and treated with 0.1% mercuric chloride (HgCl2) for 10 min followed by 4-5 times rinsing with sterile distilled water to remove traces of HgCl2 from outer surface of shoots. Leaf sheath segments (approximately 0.5 cm) were prepared on laminar air flow cabinet aseptically from sterilized shoots using a fine sterile forceps and scalpel and cultured as explants on MS medium supplemented with appropriate hormones for callus, shoot and root production. The explants were aseptically cultured on MS medium supplemented with 2, 4-D (3.0 mgl^{-1}) and coconut water (10%) for callus induction. The developed callus masses were sub-cultured on same medium at 25-30 d intervals. Then the calli were transferred to MS medium supplemented with 2.0 mgl^{-1} BAP and 1.0 mgl^{-1} Kn for shoot regeneration. The shoots, regenerated from callus were transferred to rooting medium supplemented with NAA 5.0 mgl^{-1} for root formation. For the development of salt tolerance in sugarcane variety Isd 16 every medium in every stage stressed with various levels (50, 100, 150 and 200 mM) of NaCl salt along with a salt free medium (check). Before addition of agar (0.6%), pH of media were adjusted to 5.7 and then autoclaved under 1.2 kgcm-2 pressure at 121 0C for 15 min. All cultures were incubated at 25±2 0C and kept 16 h under fluorescent tube light. The experiment was laid out following Completely Randomized Design (CRD) and replicated thrice where twenty test tubes of each treatment were maintained for observation. The plantlets were successfully transferred to earthen pots having soils thoroughly mixed with rotten pressmud at a ratio 3 : 2 and kept under polythene shed for hardening. After hardening, acclimatized plantlets were ready for field planting.

RESULTS AND DISCUSSION

The calli produced from explants were cultured on MS medium supplemented with 3.0 mgl-1 2,4-D within 21 d after inoculation. For satisfactory callus induction in sugarcane explants on MS medium supplemented with 3.0 mgl-1 2,4-D was reported optimum in this laboratory (Alam et al., 2003; Karim *et al.*, 2002; Rahman et al., 2001; Hossain et al., 1996). For development of salt tolerance in sugarcane variety Isd 16, explants were cultured on MS medium supplemented with 3.0 mgl-1 2, 4-D and NaCl salt concentrations viz. 50 , 100 , 150 and 200 mM along with a check (without salt). Callus induction was affected by NaCl salt concentrations. The callogenic response of explants declined when NaCl salt concentration was increased. The highest percentage of explants responded to callus formation was 90% when MS medium was not supplemented with NaCl salt followed by 50 mM of NaCl salt (Table 1). The lowest percentage of explants responded to callus formation was 60 at 200mM NaCl salt concentration. With the increase of NaCl salt concentration the frequency of callus induction decreased (Fig. 1). Tanvir *et al.* (2001) used 10 varieties of sugarcane for development of salt tolerance somaclones with different NaCl concentrations (0.0,0.03, 0.6, 0.9 and 1.2 gl-1), and reported that with the increase of NaCl salt concentration the response of explants to callus induction decreased. Poor callus formation was recorded from 150 and 200 mM of NaCl salt concentrations.

For shoot regeneration and multiplication the MS medium containing BAP 2.0 mgl-1 + Kn1.0 mgl-1 was used. The NaCl salt concentration have significant effects on total shoots and usable shoots production (Table 2). The lowest number of shoots and usable shoots per culture was obtained in the highest concentration of NaCl salt. The number of shoots and usable shoots per culture were found to be increased with decrease of NaCl salt concentration (Fig 2). Significant differences for shoots and usable shoots production per culture was recorded between 100 and 150 mM NaCl salt concentration levels (Table 2) , while insignificant difference in total shoots and usable shoots production between 50mm NaCl salt concentration and check (Table 2).

Multiple shoots were isolated and individually cultured on MS medium supplemented with NAA 5.0 mgl-1 and NaCl salt. Decreasing trend of root formation from shoot was observed with the increase of NaCl salt concentration in the medium (Table 3; Fig 3). Root production from shoots was found to be possible up to 100 mM of NaCl salt used in the rooting media. Similar results were also reported by several workers earlier (Liu *et al.*, 1972; Bhansali and Kishan, 1982; Shaheen and Mirza, 1989.). In vitro healthy and well established rooted plantlets were transferred to earthen pot having soils thoroughly mixed with rotten pressmud at a ratio 3: 2 kept under hardening shed for 7-14 d. After hardening, plantlets survived successfully on the soil. Successful regeneration of rooted plantlets on media supplemented with 50-100 mM NaCl salt concentrations indicates development of partial salt tolerance in the sugarcane variety Isd 16.

Table 1. Effects of NaCl salt concentration on callus induction in explants of sugarcane variety Isd 16.

Treatments	Response of explants to callus formation (%)
Check	90
MS + NaCl 50 mM	85
MS + NaCl 100 mM	75
MS + NaCl 150 mM	65
MS + NaCl 200 mM	60

Table 2. Effects of media compositions on shoot production in sugarcane variety Isd 16.

Treatments	Total number of shoots	Number of usable shoots
Check	12.1a*	7.2a
MS + NaCl 50 mM	11.5a	6.6a
MS + NaCl 100 mM	9.8b	5.8b
MS + NaCl 150 mM	8.1c	3.5c

* Figures in the columns followed by different letters are significantly different by DMRT at p= 0.05.

Table 3. Effects of media compositions on root production in sugarcane.

Treatments	Number of roots per shoot
Check	12.1a*
MS + NaCl 50 mM	11.4a
MS + NaCl 100 mM	7.2b

* Figures in the columns followed by different letters are significantly different by DMRT at p= 0.05

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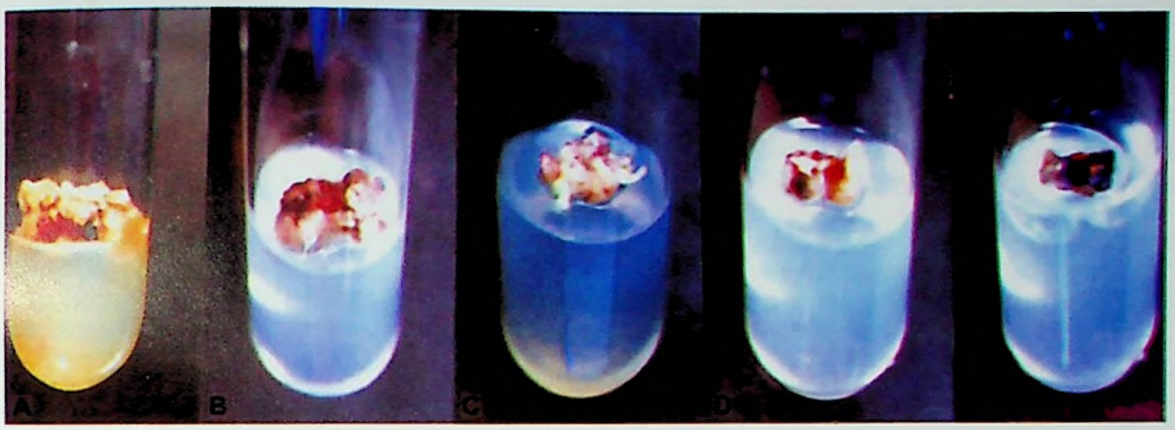

Figure 1. Effects of NaCl concentrations (A, B, C, D and E are 0, 50, 100, 150 and 200 mM respectively) on callus induction in sugarcane variety lsd 16.

Figure 2. Effects of NaCl concentrations (A, B, C and D are 0, 50, 100 and 150 mM respectively) on shoot formation in sugarcane variety lsd 16.

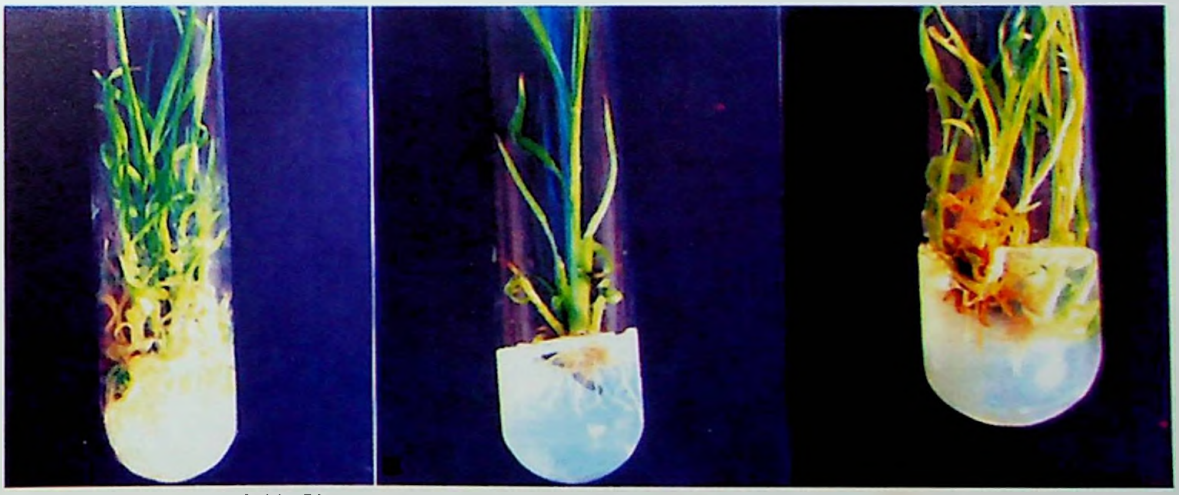

Figure 3. Effects of NaCl concentrations (A, B and C are 0, 50 and 100 mM respectively)on root formation in sugarcane variety lsd 16.