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Regeneration Potentiality of Sugarcane Variety Isd 28 Against Salt Stress Condition

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ABSTRACT

The present study was conducted to see the regeneration potentiality of sugarcane variety Isd 28 against NaCl salt stress. The unexpanded spindle leaf sheaths were used as explants. The MS medium stressed with various NaCl levels (50, 100, 150 and 200 mM) along with a check was used. The explants were aseptically cultured on MS medium supplemented with 2, 4-D ($3.0mgl^{-1}$) and coconut water (10%) for callus induction. Callus growth was decreased with the increased concentration of salt. The growth regulator BAP $2.0mgl^{-1}$ and Kn $1.0mgl^{-1}$ with MS medium were used for shoot regeneration and multiplication. The MS medium supplemented with NAA 5.0 mgl^{-1} was used for *in vitro* root formation from proliferated shoots. When the medium was supplemented with different concentrations of NaCl salt, shoot formation and multiplication, and root producing capacity decreased with the increase in salt concentration. At 200mM of NaCl salt most of the calluses became black and finally died within 35 days of incubation. Regeneration of plantlets with sufficient roots on medium supplemented with 50 and 100 mM NaCl salt indicates that sugarcane variety Isd 28 deserves partial regeneration potentiality against NaCl salt stress.

Key words: Sugarcane, Isd 28, regeneration potentiality, NaCl salt stress.

INTRODUCTION

Like many other countries of the world, soil salinity has severely affected the agricultural productivity of Bangladesh. Sugarcane is a principal cash crop in North-Western and South-Western low rainfall belt of the country and the main raw material for sugar and gur industries. One of the main causes of low yield in sugarcane is the vulnerability of the crop to environmental stresses. Among the environmental stresses, salinity is one of the major problem in reducing crop yield. Sugarcane cultivation in saline areas received no research attention in the past. But due to increased demand of sugar and gur for local consumption it has become imperative to explore potentials of salt stress prone lands where sugarcane is being cultivated years together without adopting modern technologies. To meet the future requirement of sugar it is essential to develop some improved varieties, suitable for the stressful condition of Bangladesh within shorter period of time.

There is no rapid method to develop a variety within shorter period of time using conventional breeding method. The sugarcane breeding programme has been under

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serious problem due to lack of suitable multiplication procedure (Lal and Sing 1994). Conventional breeding method usually required 10-15 years of work to complete a selection cycle for release of a variety and an important variety can be planted commercially several years later when enough seed canes will have been produced. Time required and continued contamination by systemic disease are serious problem to multiply a elite genotype of sugarcane in the open field (Lal and Sing 1994). 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 breeding and clonal propagation methods.

It should be mentioned that Krsishnamurthi (1974) was the first to obtain Fiji and Downy mildew disease resistance sugarcane from variety Pindar. Then Eyespot, Smut, Leaf scald diseases and Mosaic resistance somaclones were developed by Heinz et al. (1977), Liu (1981), Birch (1997) and Joyce et al. (1997) respectively. Varietal development of sugarcane for salt tolerance was reported by many workers (Tanvir et al., 2001; Liu and Yeh, 1992; Fitch and More, 1981).

The alternative approach of utilizing potential plant cells for the development of salt tolerance in sugarcane has received increased research attention in the 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 compartmentalization of excess salt in the vacuoles and survival 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). The experiment was made to see the regeneration potentiality of sugarcane variety Isd 28 against salt stress and determine the critical limit of NaCl concentration for different *in vitro* stages.

MATERIALS AND METHODS

The sugarcane variety Isd 28 were collected from research farm of Bangladesh Sugarcane Research Institute and the experiment was conducted at Biotechnology Laboratory of this Institute. Tops (20-30 cm long) of 3-5 months old field grown sugarcane plants of variety Isd 28 were collected and the leaf explants, 5cm in length, were excised. Excision of explants was carried out under aseptic conditions with sterilized tools. The explants 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 ($HgCl_2$) for 10 min followed by 4-5 times rinsing with sterile distilled water to remove traces of $HgCl_2$ from outer surface of shoots. Leaf sheath segments (approximately 1cmx0.5 cm) were prepared in laminar air flow cabinet aseptically from sterilized shoots and cultured as explants on MS medium supplemented with appropriate hormones for regeneration of callus, shoot and roots. For callusing 2, 4-D (3.0 mgl^{-1}) and coconut water (10%) were used in MS medium. Callus masses so developed were sub-cultured for 3 times successively on

fresh MS medium having same NaCl levels as used for callus initiation at 30 d interval. Healthy callus of each treatment was transferred to regeneration medium i.e. MS medium supplemented with 2.0 mgl^{-1} BAP and 1.0 mgl^{-1} Kn. The shoots regenerated from callus were transferred to rooting medium (MS + NAA 5.0 mgl^{-1}) for root induction. In every stage MS medium stressed with various levels (50, 100, 150 and 200 mM) of NaCl salt along with a check were used. The pH of media were adjusted to 5.7. Agar, 0.6%, were added to the medium. All media were sterilized by autoclaving at 1.2 $kgcm^{-2}$ pressure at 121 ^0C for 15 min. Cultures were incubated at 25±2 ^0C and kept 16 h under fluorescent tube light. The experiment was laid out in Completely Randomized Design (CRD).There were three replication and twenty test tubes of each treatment were maintained for observation.

RESULTS AND DISCUSSION

In the present investigation, the techniques of *in vitro* plant regeneration through callus induction followed by shoots regeneration, multiplication and rooting in shoots under salt stressed conditions have carefully been done. The foremost among these is an excellent callus culture and plant regeneration system which is simple and reproducible to apply. It has been found effective for virtually all sugarcane cultivars (Heinz et al., 1977; Ho and Vasil, 1983; Chen et al., 1988; Taylor, 1992) was adopted.

For callus induction from leaf sheath explant of variety Isd 28 2,4-D 3.0 mgl^{-1} was used with NaCl salt concentrations of 50 mM, 100 mM, 150 mM, 200 mM and 200 mM with a control treatment without salt. Callus induction was affected by NaCl salt concentrations. Response of explant to callus initiation declined when NaCl salt was added to MS medium. The highest percentage of explants responded to callus formation was 90% in the variety Isd 28 when MS medium was supplemented with 50 mM of NaCl salt. With the increase in NaCl salt concentrations the frequency of callus induction was decreased. The lowest percentage of explants responded to callus formation was 65% (Table 1). Tanvir *et al.* (2001) used 10 varieties of sugarcane for development of salt tolerance somaclones using NaCl salt (0.0, 0.03, 0.6, 0.9 and 1.2 gl^{-1}) and reported that with the increase of NaCl salt concentrations decreased the response of explants to callus induction. High salinity causes hyperosmotic stress and ion disequilibrium that produce secondary effects (Zhu, 2001; Hasegawa et al., 2000). Decrease of callus formation response of explants was found in salinity stress plausibly due to higher osmotic stress exerted by NaCl salt in the medium.

Table 1. Effects of NaCl salt concentration on callus induction in explants of sugarcane variety Isd 28.

Treatments	Response of explants in callus formation (%)
Control	95
MS + NaCl 50 mM	90
MS + NaCl 100 mM	85
MS + NaCl 150 mM	85
MS + NaCl 200 mM	85

Poor callus formation was recorded from 150 and 200 mM of NaCl salt concentration in variety Isd 28 .With the increase of salt concentrations the callus became reddish black in colour. In the medium where 200 mM of NaCl salt was used the callus became black and died after 35-40 days of incubation (Table 2, Fig.1)

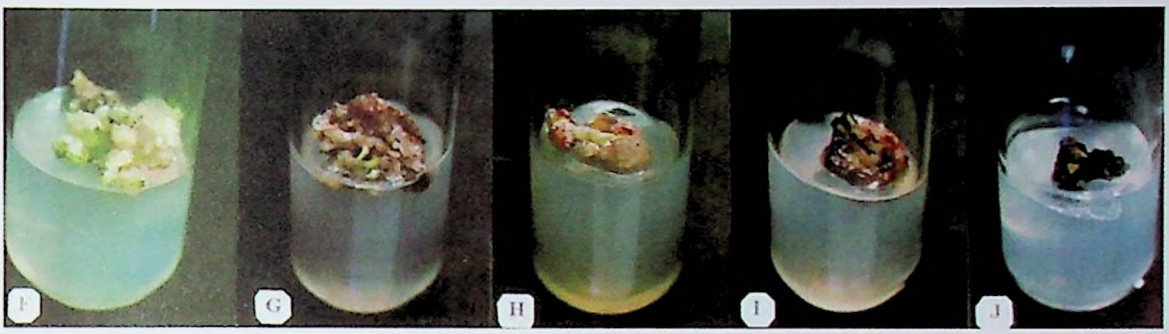

Fig. 1 Effect s of NaCl concentrations on callus induction in sugarcane variety Isd 28 on media containing 0, 50, 100,150 and 200 mM of NaCl.

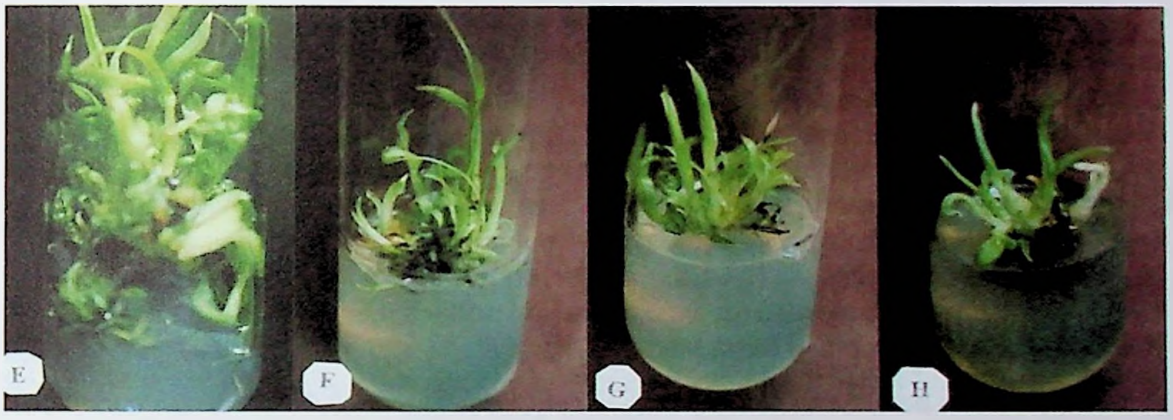

Fig. 2 . Effects of NaCl concentrations on shoots formation in sugarcane variety Isd 28 on media containing 0, 50, 100 and 150mM of NaCl.

Fig. 3. Effects of NaCl concentrations on roots formation in sugarcane variety Isd 28 on media containing 0, 50 and 100mM of NaCl.

Table 2. Effects of NaCl salt concentration on development of callus in sugarcane variety Isd 28.

Treatment	Isd 28
Control whitish	Vigorous
MS + NaCl 50 mM Reddish	Vigorous
MS + NaCl 100 mM reddish	vigorous
MS + NaCl 150 mM reddish	poor
MS + NaCl 200 mM	Poor reddish-black and dead

The results of shoot formation and multiplication have been presented in Table 3. The higher number of shoots and usable shoots per culture was obtained where lower concentration of NaCl salt was used (Table 3). The number of shoots and usable shoots per culture was decreased with the increase in NaCl salt in the sugarcane variety Isd 28. Multiple shoots were isolated and individually cultured on MS medium supplemented with NAA 5.0 mgl^{-1}. The results of root production are shown in Table 4. Number of roots per shoot was decreased with the increase in NaCl salt concentration in the medium indicating that the root production capacity decreased with the increase of NaCl salt concentration. Root production in shoots was possible up to 100 mM of NaCl salt used in the rooting media. Similar results were also reported by several workers (Liu et al., 1972; Bhansali and Kisahan, 1982; Shaheen and Mirza, 1989; Shaid et al., 1990).

Regeneration potentiality of sugarcane variety Isd 28 under NaCl salt stress conditions indicates the possibility of sugarcane variety development for cultivation under salinity stress prone areas of Bangladesh.

Table 3. Major effects of media compositions on shoot production in sugarcane variety Isd 28.

Treatment	Total number of shoot	Number of usable shoot
Control	12.08a*	7.21a
MS + NaCl 50mM	11.46a	6.58a
MS + NaCl 100mM	9.79b	5.75b
MS + NaCl 150mM	8.04c	3.50c

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

Table 4. Major effects of media compositions on root production in sugarcane variety Isd 28.

Treatment	Number of roots per shoot
Control	12.14a*
MS + NaCl 50 mM	11.36a
MS + NaCl 100 mM	7.21b

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

REFERENCES

Bhanshali, R R and Kishan, S. 1982. Callus and shoot formation from leaf of sugarcane in tissue culture. *Phytomorphology*, **32** (2/3) : 167-170.

Birch, R.G.1997.Transgenic sugarcane: opportunities and limitations. *In:* Keating B.A. and Wilson, J.R.(EDs) Intensive Sugarcane Production: Meeting the Challenges Beyond 2000. CAB Intl. Wallinngford, UK.pp.125-140

Chandler, S F and Thrope, T A. 1986. Variation from plant tissue culture: biotechnological application to improving salinity tolerance. *Biotechnol. Adv.*, **4**: 117-135.

Chen, W.H.; Davey, M.R.; Power, J.B. and Cockong, E.C.1988.Control and maintenance of plant regeneration in sugarcane callus culture.*J.Exp.Bot.* **39**: 251.

Fitch, M.M. and Moore, P.H. 1981. The selection for salt resistance in sugarcane (*Saccharum* spp. hybrids) tissue culture. *Plant Physiol.* **67** (No. 6, suuppl.): 26 (Abstr).

Heinz, D.J.; Krisnamurti, M.; Nickell, L.G. and Maretzi, A.1977. Cell tissue and organic culture in sugarcane improvement. In : Applied and Fundamental Aspects of Plant Cell, Tissue and Organ Culture.(Eds. Reinert J. and bajaj Y.P.S.), Springer, Berlin, Heidelburg, New York,pp.3-17.

Ho, W.J. and Vasil, I.K. 1983. Somatic embryogenesis in sugarcane (*Saccharum officinarum* L.). The morphology of callus formation and the ontogeny of somatic embryos. Protoplasma, **118**: 169-180.

Hasegawa, P.M.; Bressan, R.A.; Zhu, J.K. and Bohnert H.J.2000.Plant Cellular and molecular responses to high salinity. Annu. Rev. Plant Physiol. *Plant Mol.Biol.* **51**: 463-499.

Joyce, P.A.; McQyalter, R.B.; Bernard, J.J.and Smith, G.R.1997. Engineering for resistance to SCMV in sugarcane. Acta Hort., **461**: 385-391.

Krisnamurti, M. 1974. Notes on disease of tissue culture subclones fusion of sugarcane protoplasts.ISSCT Sugarcane Breed. News l., 35: 24-26.

Liu, M.C. 1981.*In vitro* method applied to sugarcane improvement and application in agriculture academic press, pp: 191-323.

Liu, M.C. and Yeh, H.S.1992. Selection of NaCl tolerant line through stepwise salanized sugarcane cell culture. In: Proceedings 5th Intl. Cong. Plant Tissue and cell culture 1982.(A. Fujiwara, ed) .pp: 477-478. Japanese Association of Plant Tissue Culture,Tokyo.

Liu, M C; Huang, Y J and Shih, S C. 1972. The *in vitro* production of plants from several tissues of *Saccharum* spp. *J. Agric. Ass.* China. **77** : 52-58.

Lal, N. and Singh, H.N. 1994. Rapid clonal multiplication of sugarcane through tissue culture. Plant Tissue Cul. **4** (1): 1-7.

Shaheen, M S and Mirza, M S. 1989. *In vitro* production of plants from sugarcane tissue. *J. Agric.* Res. **26** (6) : 302-312.

Staverek, S J and Rains, D W. 1984. The development of tolerance to mineral stress. *Hort. Sci.* **19** : 337-382.

Shaid, M.T.H.; Shaheen, M.S. and Mirza, M.S.1990. Studies on comparative response of sugarcane varieties to callus production. Processing of "National Seminar on Sugarcane Production" held on January.

Tanvir, M K; Ali, R; Akhtar, S; Rahman, S and Choudhury, B A. 2001. Development of salt tolerant sugarcane plants through tissue culture. *Pak. Sugar* J. **16** (6): 32-35.

Taylor, P.W.J.1992. Micropropagation of sugarcane (*Saccharum spp.*hybrid) .Biotech. Agric. Forest. **39**:256-268.

Zhu, J.K. 2001.Plant salt tolerance, *Trend in Plant* Sci. **6**: 66-71.