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Regeneration of sugarcane variety ISD 40 against salt stress condition

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ABSTRACT

The experiment was conducted at the Biotechnology Laboratory, Bangladesh Sugarcane Research Institute (BSRI), Ishurdi, Pabna, Bangladesh in 2013. The effects of MS medium supplemented with different salt (NaCl) concentrations (190, 200, 210, 220 and 230 mM) containing 2,4-D (3 mg/l) along with green coconut water (10%) on explants (leaf sheath) were found out in sugarcane variety Isd 40. Besides, regeneration potentiality including shooting and rooting ability under different levels of NaCl (190, 200, 210, 220 and 230 mM) was investigated. MS medium supplemented with NaCl (190 mM) containing 3.0 mg/ l 2, 4-D along with green coconut water (10%) produced the highest callus induction (91.67 %) followed by (81.67%) MS medium supplemented with NaCl (200 mM) containing 3.0 mg/ l 2, 4-D along with green coconut water (10%) among five levels of concentration (Salt). The lowest days to callus

initiation (23.84) was found in MS medium supplemented with NaCl (190 mM) containing 3.0 mg/ l 2, 4-D along with green coconut water (10%) while the highest days to callus initiation (31.92) was found in MS medium supplemented with NaCl (230 mM) containing 3.0 mg/l 2, 4-D along with green coconut water (10%). The highest shoot and root regeneration (70.67% and 64.00%) were obtained from MS medium fortified with 190 mM NaCl containing combination of BAP and Kn (2 mg/l + 1 mg/l) while the lowest shoot and root regeneration (53.33% and 20.63%) respectively from MS medium supplemented with 230 mM salt containing combination of BAP and Kn (2 mg/l + 1 mg/l). However, NaCl had significant effects on callus, shoot and root formation. It revealed that numbers of callus, shoot and root were decreased by increasing of NaCl concentration levels.

Key words: Callus, 2, 4-D, NaCl, Regeneration, Sugarcane, Salt tolerant.

1. INTRODUCTION

Sugarcane is globally the main source of raw material for the production of sugar. Although many countries are producers, only six of them account for 65% of the world's entire sugarcane production. Among these Brazil is the largest one (Viera, 2002). Sugarcane is a tropical glycophyte exhibiting stunted growth or no growth under salinity, with its yield failing to 50% or even more its true potential (Subbarao and Shaw, 1995) which could possibly be due to the accumulation of toxic ions. Besides, plants responses to salt stress are complex involving many genetic networks and metabolic processes and these depend on the inherent salt tolerance of the plant (Karpe *et al.,* 2012). Tissue culture offers an opportunity to mass produce disease free planting material and is now used to supplement commercial sugarcane propagation in many countries including Brazil, the United States, India and Cuba (Lakshmanan *et al.,* 2006). It is a principal cash crop in north western and south western low rainfall belt of the country. Salinity is the most serious problem effecting the agricultural production in Bangladesh. Sugarcane is extremely salt sensitive crop and soil salinity is a major constraint in the production of sugarcane in southern region of Bangladesh. In 1973 the salinity area was 0.83 million hectare (mha), in 2000, it was 1.02 mha and in 2009 it became 1.06 mha. Saline soils have a high content of soluble salts. In this soil, electrical conductivity (EC) of saturated extract is more than 4 ds/m at 25^0C, exchangeable sodium percentage (ESP) value is less than 15 and the pH value is below 8.5. It is also called white alkali soils. The soluble salts are mostly chlorides and sulphates of Na, Ca and Mg. The salinity increases in dry months showing a peak in March-April and decreases in wet months with the minimum in July-August (Hassan *et al.* 2012). Abiotic stresses like salt and drought induce changes in morphological, metabolites and molecular attributes that adversely affect plant growth and productivity (Mahajan and Tuteja, 2005). Plant adaptations to salinity are of three distinct types: osmotic stress tolerance; Na+ exclusion; and tissue tolerance, that is tolerance of tissue accumulated Na+ and possibly Cl- (Munns and Tester, 2008). The low cane and sugar yields are attributed to many factors in which drought, salinity, insect pests and diseases are major constraints (Nasir *et al.,* 2000 and Khaliq *et al.,* 2005). High concentrations of salts in soils account for large decreases in the yield of a wide variety of crop all over the world (Tester and Davenport, 2003). In Bangladesh, about o.833 million hectares of available lands of southern districts are affected by salt. The present situation demands for cultivation of salt tolerant sugarcane variety in northern districts of Bangladesh. Due to increased demand of sugar and gur for local consumption, 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 along with salt tolerant by tissue culture. Although, sugarcane is one of the most important industrial crops, very limited effort have been made on tissue culture and *in vitro* propagation for salt tolerant variety development and multiplication of Bangladesh. Hence tissue culture research using modern sugarcane variety of Bangladesh deserve due attention. Therefore, this experiment was conducted to find out salt tolerant callusing, shooting and rooting potentiality as well as developing of salt tolerant sugarcane variety along with increased salt tolerance level by applying NaCl.

2. MATERIALS AND METHODS

The experiment was conducted at the Biotechnology Laboratory, Bangladesh Sugarcrop Research Institute (BSRI), Ishurdi, Pabna, Bangladesh in 2013 to obtain *in vitro* salt tolerant plant regeneration potentiality of BSRI released variety Isd 40. The experimental materials were the young leaf sheath of BSRI released variety Isd 40. The explants were collected from 8-10 months old field grown sugarcane from BSRI experimental field. MS medium supplemented with different salt (NaCl) concentrations (190, 200, 210, 220 and 230 mM) containing 2, 4-D (3 mg/l) along with green coconut water (10%) on explants (leaf sheath) were used as treatment for callus induction. Induced calli were inoculated on shooting medium (2 mg/l BAP + 1 mg/l) under different levels of NaCl (190, 200, 210, 220 and 230 mM) for shoot formation. After shoots formation, shoots were inoculated on rooting medium NAA (5 mg/l) under different levels of NaCl (190, 200, 210, 220 and 230 mM) for root formation. Mediums were adjusted to pH (5.8). Agar (0.6%) was added medium. All media were sterilized by autoclaving at 1.2 Kg cm^{-2} pressure at 121° C for 30 minutes. Mercuric Chloride ($HgCl_2$)

was used as sterilizing agent while savlon was used as antiseptic, detergent and surfactant. The explants were taken in a beaker and treated with 1% (w/v) savlon for 5-6 minutes with constant shaking and washed thoroughly with distilled water for 3-4 minutes. The explants were transferred in autoclaved conical flask (500 ml) treated with 0.1% mercuric ($HgCl_2$) for 10 minutes and washed by 3-4 times rinsing with sterile distilled water to remove traces of $HgCl_2$ from outer surface of leaf sheath segments. Explants (approximately 1 cm x 0.5 cm) were prepared in laminar air flow cabinet from sterilized leaf sheath segments. Cultures were incubated at 25±2ºC and kept 16h under fluorescent tube light. The experiment was laid out in Completely Randomized Design (CRD). Three replications and ten test tubes of each treatment were maintained for observation. The data for the characters under the present study were statistically analyzed following Completely Randomized Design (CRD). The analysis of variance was performed by Least Significant Difference (LSD) test at 5% level of probability for interpretation of result (Gomez and Gomez, 1984).

3. RESULTS AND DISCUSSION

Different concentrations of Nacl had significant effects on callus, shoot, root induction etc (Figure 1, 2 & 3 Table 1, 2 & 3). When Isd 40 sugarcane variety explants (leaf sheath) were inoculated on MS medium supplemented with different salt (NaCl) concentrations (190, 200, 210, 220 and 230 mM) containing 2,4-D (3 mg/l) along with green coconut water (10%), the callus induction (91.67%) was obtained from MS medium containing NaCl (190 mM) supplemented with 2, 4 – D 3 mg/l along with green coconut water (10%) followed by MS medium containing NaCl (200 mM) supplemented with 2, 4–D 4 mg/l (81.67%) along with green coconut water (10%) (Table 1, Figure 1 & 2). On the other hand, the lowest callus induction (52.67%) was produced from MS medium containing NaCl (190 mM) supplemented with 2, 4 – D 3 mg/l along with green coconut water (10%) among five levels of concentration (Salt). The lowest days requirement to callus initiation (23.84) was found in MS medium supplemented with NaCl (190 mM) containing 3.0 mg/l 2, 4-D along with green coconut water (10%) while the highest days requirement to callus initiation (31.92) was found in MS medium supplemented with NaCl (230 mM) containing 3.0 mg/l 2, 4-D along with green coconut water (10%). The highest shoot and root regeneration (70.67% and 64.00%) were recorded from MS medium fortified with 190 mM NaCl containing BAP 2 mg/l + Kn 1 mg/l and MS medium fortified with NaCl (190 mM) containing NAA (5 mg/l) while the lowest shoot and root regeneration (53.33% and 20.63%) from MS medium supplemented with 230 mM salt containing BAP 2 mg/l and Kn 1 mg/l and MS medium fortified with 230 mM NaCl containing NAA 5 mg/l (Table 2 and 3, Figure 2 and 3). Furthermore, the lowest days requirement to shoot and root initiation (7.99 and 15. 67) were showed in MS medium fortified with 190 mM NaCl containing BAP 2 mg/l + Kn 1 mg/l and MS medium fortified with NaCl (190 mM) containing NAA (5 mg/l) while the highest days requirement to shoot and root initiation (10.67 and 19.33) were observed in MS medium fortified with 230 mM NaCl containing BAP 2 mg/l + Kn 1 mg/l) and MS medium fortified with NaCl (230 mM) containing NAA (5 mg/l) respectively. Besides, the maximum number of shoots per callus (7.417) and roots per shoot (4.8) were found from MS medium fortified with 190 mM NaCl containing BAP 2 mg/l + Kn 1 mg/l and MS medium fortified with NaCl (190 mM) containing NAA (5 mg/l) while the minimum number of shoots per callus (4.49) and roots per shoot (1.13) were observed in MS medium fortified with 230 mM NaCl containing BAP 2 mg/l + Kn 1 mg/l) and MS medium fortified with NaCl (230 mM) containing NAA (5 mg/l) respectively. This result is in agreement with the finding of Mahmud et al. 2012. Finally regenerated plantlets were transplanted in the polybag and then field after hardening (Figure 4, 5 and 6). It revealed that numbers of callus, shoot and root regeneration were decreased by increasing of NaCl concentration levels.

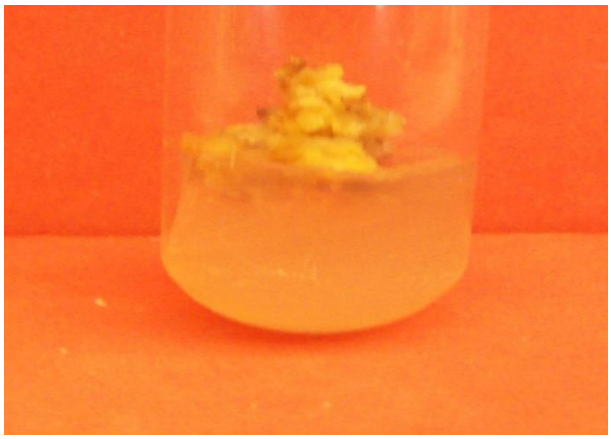

Figure 1 Regeneration of salt tolerant callus at 190mM level of NaCl

Figure 2 Regenerated salt tolerant shoot at190mM level of NaCl

Figure 3 Regenerated salt tolerant root at190mM level of NaCl

Figure 4 Regenerated salt tolerant plantlet was transplanted in the polybag

Figure 5 Regenerated salt tolerant plantlets in hardening shade

Figure 6 Regenerated salt tolerant somaclone in the field

Table 1 Effects of Nacl under different concentrations on callus induction in Sugarcane

Treatments	Callus induction (%)	Days required to callus initiation
190mM NaCl	91.67 a	23.84 e
200mM NaCl	81.67 b	25.23 d
210mM NaCl	69.00 c	26.63 c
220mM NaCl	66.33 d	29.58 b
230mM NaCl	52.67 e	31.93 a
LSD at 5% level	2.048	0.874

Figure in the column with same letter do not differ significantly at 5% level of probability as per Duncan's Multiple Range Test (DMRT)

Table 2 Effects of NaCl under different concentrations on shoots formation in sugarcane

Treatments	Days required to shoot initiation	Number of shoot per callus	Shoot regeneration %
190mM NaCl	7.99 e	7.417 a	70.67 a
200mM NaCl	8.58 d	7.107 b	64.00 b
210mM NaCl	9.10 c	6.76 c	61.67 c
220mM NaCl	9.78 b	5.64 d	58.33 d

230mM NaCl	10.67 a	4.49 e	53.33 e
LSD at 5% level	1.081	0.244	2.048

Figure in the column with same letter do not differ significantly at 5% level of probability as per Duncan's Multiple Range Test (DMRT)

Table 3 Effects of NaCl under different concentrations on roots formation in sugarcane

Treatments	Days required to root initiation	Number of root per shoot	Root regeneration %
190mM NaCl	15.67 c	4.8 a	64.00 a
200mM NaCl	16.00 c	4.00 b	57.74 b
210mM NaCl	17.66 b	3.80 b	52.46 c
220mM NaCl	18.33 ab	2.06 c	42.26 d
230mM NaCl	19.33 a	1.13 d	20.63 e
LSD at 5% level	1.266	0.3172	

Figure in the column with same letter do not differ significantly at 5% level of probability as per Duncan's Multiple Range Test (DMRT)

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