Bangladesh J. Sugarcane, 35 : 73-78 *June, 2014*

Response of 2, 4-D on Callus Induction and Plantlet Regeneration in Sugarcane

K. Mahmud[1*], K.M. Nasiruddin[2], M.A. Hossain[3] M.A. Rahman[4] and A.K. Ghose[1]
[2]Prof. Dept. of Biotechnology, Bangladesh Agricultural University, Mymensingh
[1]Biotechnology Division, [4]On-farm research Division, [3]Director (Research)
Bangladesh Sugarcrop Research Institute, Ishurdi-6620, Pabna, Bangladesh

ABSTRACT

The experiment was conducted at the Biotechnology Laboratory, Bangladesh Sugarcane Research Institute (BSRI), Ishurdi, Pabna, Bangladesh. The effects of only MS medium, MS medium with green coconut water (10%, 20%, 30%, 40% and 50%) and MS medium with green coconut water containing various concentration (1.0, 2.0, 3.0, 4.0 and 5.0 mg l^{-1}) of 2, 4-D on callus induction, shoot and root formation of sugarcane variety Isd 40 were investigated. No callus, shoot and root were produced in only MS medium and MS medium with green coconut water (10%, 20%, 30%, 40% and 50%). But MS medium containing green coconut water supplemented with different concentrations of 2, 4-D (1.0, 2.0, 3.0, 4.0 and 5.0 mg l^{-1}) produced callus, shoot and also root. The frequency of callus induction varied from 76.67 to 100 % depending on the different concentrations of 2, 4-D. Among the callus induction medium containing 3.0 mg l^{-1} 2, 4-D was found to be the best for callusing (100%). After callus formation a few number of shoots and roots were regenerated directly from some callus. The highest shoots (7.0 per callus) and roots (4.0 per callus) were produced from MS medium containing green coconut water supplemented with 1 mg l^{-1} 2, 4-D. On the other hand, the lowest shoots (1.33 per callus) were produced from MS medium with green coconut water containing 5 mg l^{-1} 2, 4-D. Besides, no roots were produced from MS medium with green coconut water containing 4 and 5 mg l^{-1} 2, 4-D. The lowest day's requirement for induction of callus (12.00) and shoot (21.33) were obtained from MS medium with green coconut water containing 1 mg l^{-1} 2, 4-D. Therefore, it may be recommended that 3.0 mg l^{-1} 2, 4-D may be used for only callus induction but 1.0 to 3.0 mg l^{-1} 2, 4-D for callusing with shooting and rooting in sugarcane variety Isd 40.

Key words: Callus, 2, 4 - D, root and shoot regeneration, sugarcane

INTRODUCTION

Sugarcane is globally the main source of raw material for the production of sugar. It 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 goor industries. The genus *Saccharum* contains three cultivated (*S. officinarum*, *S. sinensis* and *S. barbari L*) and two wild (*S. robustum* and *S. spontanium*) species (Kochhar, 1998). The geographical origin of modern cultivated sugarcane (*S. officinarum*) is New Guinea and distributed throughout the tropics and sub-tropics are generally agreed. Sugarcane plant regenerated from

* Corresponding author: K. Mahmud, Principal Scientific Officer
e-mail: kmahmud31@yahoo.com

tissue and cell culture show heritable variation for both qualitative and quantitative trait and considered as a powerful tool for crop improvement within limited time period. For the purpose development of regeneration protocols is prerequisite. Leaf tissue of modern hybrids of sugarcane clones is usually more responsive to tissue culture than those of traditional varieties or wild relatives (Chen *et al.*, 1998; Taylor *et al.*, 1997). The sugarcane breeding programme has been under 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 section 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 contaminations by systemic diseases 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 the clonal propagation methods. Due to increased demand of sugar and goor 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 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 variety development and multiplication of Bangladesh. Hence tissue culture research using modern sugarcane varieties of Bangladesh deserves due attention. Therefore, this experiment was conducted to find out direct callusing with shooting and rooting potentiality of sugarcane variety Isd 40 by applying growth regulator hormone 2, 4-D.

MATERIALS AND METHODS

The experiment was conducted at the Biotechnology Laboratory, Bangladesh Sugarcane Research Institute (BSRI), Ishurdi, Pabna, Bangladesh during the period from September, 2010 to January, 2011 to obtain *in vitro* plant regeneration potentiality of BSRI released variety Isd 40 (Figure 1). The experimental materials was 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. Only MS medium, MS medium with coconut water (10%, 20%, 30%, 40% and 50%) and MS medium with green coconut water (10%) containing five concentrations of 2, 4-D (1, 2, 3, 4 and 5 mg l^{-1}) were used as treatment for callus induction, shoot and 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% ($HgCl_2$) 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 and cultured on only MS medium, MS medium with coconut water and MS medium with green coconut water containing five concentrations of 2, 4-D (1, 2, 3, 4 and 5 mg l^{-1}). Cultures were incubated at 25±2°C and kept 16 hour under fluorescent tube light. The experiment was laid out in Completely Randomized Design (CRD). Three replications and ten test tubes of

of each treatment were maintained for observation. The data for the characters under the present study were statistically analyzed following 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).

RESULTS AND DISCUSSION

Different concentrations of 2, 4-D had significant effects on callus, shoot, root induction etc., (Figure 1 and 2, Table 1 and 2). When explants were inoculated on MS medium, MS medium with green coconut water (10%, 20%, 30%, 40% and 50%) and MS medium with green coconut water (10%) supplemented with 2, 4- D (1.0, 2.0, 3.0, 4.0 and 5.0 mg l^{-1}), no callus was produced on both only MS medium and MS medium with green coconut water (Figure 1). On the other hand, the highest callus (100%) was produced from MS medium containing green coconut water supplemented with 2, 4 – D 3 mg l^{-1} followed by MS medium containing green coconut water supplemented with 2, 4–D 4 mg l^{-1} (96.67%) (Table 1 & Figure 1). This finding is in agreement with the results of Alam *et al.* (2003); Karim *et al.* (2002); Hossain *et al.* (1996); Ali *et al.* (2008); Gopitha *et al.* (2010) and Mahmud *et al.* (2013). The lowest days (12.0) requirement of callus induction were obtained from MS medium containing green coconut water supplemented with 2, 4–D 1 mg l^{-1} after explants inoculation while the highest days (13.67) requirement of callus induction from MS medium containing green coconut water supplemented with 2, 4–D (4 & 5) mg l^{-1}. Besides, the highest weight (1.77 gm) $callus^{-1}$ was recorded from MS medium containing green coconut water supplemented with 2, 4–D 3 mg l^{-1} where the lowest weight (1.37 gm) $callus^{-1}$ from MS medium containing green coconut water supplemented with 2, 4–D 5 mg l^{-1}. It is revealed that shoots and roots were produced directly from callus derived from MS medium with green coconut water supplemented 2, 4-D (1.0, 2.0, 3.0, 4.0 and 5.0 mg l^{-1}) (Figure 1). The highest callus % (96.67) produced shoots from MS medium with green coconut water supplemented 2, 4-D 1.0 mg l^{-1} followed by MS medium with green coconut water supplemented 2, 4-D (2.0 & 3.0) mg l^{-1} (76.67%) while the lowest (23.33%) from MS medium with green coconut water supplemented with 2, 4-D 5.0 mg l^{-1} (Table 2). The highest number of shoots per callus (7.0) were found from MS medium containing green coconut water supplemented with 2, 4–D (1.0 & 2.0) mg l^{-1} while the lowest (1.33 shoots per callus) from MS medium with green coconut water supplemented 2, 4-D 5.0 mg l^{-1}. The lowest days (21.33) requirement of shoot induction were obtained from MS medium containing green coconut water supplemented with 2, 4–D 1.0 mg l^{-1} after callus formation while the highest days (35.67) requirement of shoot induction from MS medium containing green coconut water supplemented with 2, 4–D 5.0 mg l^{-1}. The highest number of roots $callus^{-1}$(4.0) were obtained from MS medium containing green coconut water supplemented with 2, 4–D 1.0 mg l^{-1} followed by MS medium containing green coconut water supplemented with 2, 4–D 2.0 mg l^{-1} (2.0 roots per callus). On the contrary, no root was produced by MS medium with green coconut water supplemented with 2, 4-D (4.0 & 5.0) mg l^{-1} (Table 2). Finally regenerated plantlets were transplanted in the polybag and then field after hardening (Figure 2).

Figure 1. A. Sugarcane variety Isd 40, B. No callus produced in MS medium with green coconut water (GCW), C. Callus induced from sugarcane variety Isd 40 under concentration of 3 mg/l 2, 4–D, D. Regeneration of shoots produced from leaf sheath derived calli of sugarcane variety Isd 40 under concentration of 3 mg/l 2, 4–D supplemented with 10% green coconut water

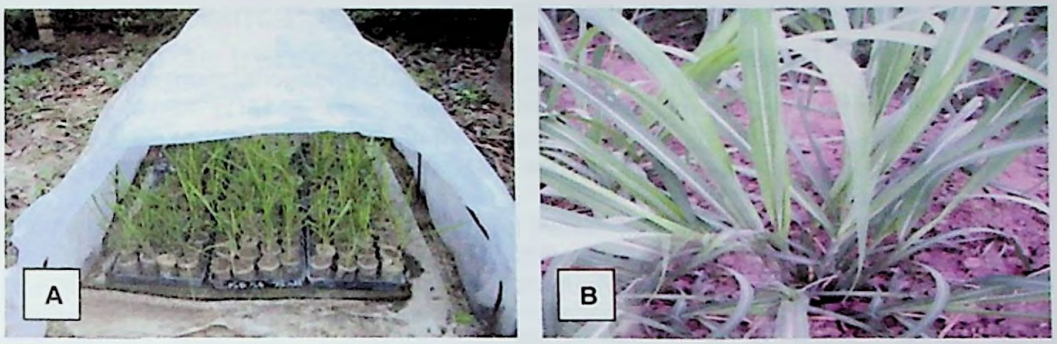

Figure 2. A. Regenerated plantlets were transplanted in the polybag and then hardening shade, B. Somaclone in the field

Table 1. **Effects of 2, 4-D on callus induction in Sugarcane**

Treatments	Callus induction (%)	Days required of callus initiation	Weight (gm)/Callus
1 mg l^{-1} 2, 4-D	76.67 b	12.00	1.43 c
2 mg l^{-1} 2, 4-D	80.00 b	12.33	1.63 b
3 mg l^{-1} 2, 4-D	100.00 a	12.67	1.77 a
4 mg l^{-1} 2, 4-D	96.67 a	13.67	1.63 b
5 mg l^{-1} 2, 4-D	76.67 b	13.67	1.37 c
LSD (0.05)	5.538	-	0.1238

Figure in the column with same letter do not differ significantly at 5% level of probability as per DMRT

Table 2. Effects of 2, 4-D on shoots and roots formation in sugarcane

Treatments	Callus % produced shoot	Number of shoot/callus	Days required of shoot initiation	Number of root/callus
1 mg l^{-1} 2, 4-D	96.67 a	7.00 a	21.33 d	4.00 a
2 mg l^{-1} 2, 4-D	76.67 b	7.00 a	21.67 d	2.00 b
3 mg l^{-1} 2, 4-D	76.67 b	5.67 b	23.33 c	1.33 c
4 mg l^{-1} 2, 4-D	53.33 c	4.00 c	24.33 b	0.00 d
5 mg l^{-1} 2, 4-D	23.33 d	1.33 d	35.67 a	0.00 d
LSD (0.05)	9.592	0.599	0.5538	0.4533

Figure in the column with same letter do not differ significantly at 5% level of probability as per DMRT

REFERENCES

Alam, R.; Mannan, S.K.; Karim, Z. and Amin, M.N. 2003. Regeneration of sugarcane (*Saccharum officinarum*) plantlet for callus. *Pak. Sugar J.*, **18**(1): 15-19.

Ali, A.; Naz, S.; Siddique, F. A. and Iqbal, J. 2008. Rapid clonal multiplication of sugarcane (*Saccharum officinarum*) through callogenesis and organogenesis. *Pak. J. Bot.*, **40**(1): 123-138.

Bhansali, R. R. and Kishan, S. 1982. Callus and shoot formation from leaf of sugarcane in tissue culture. *Phytomorphology*, pp.167-170.

Burner, D. M. and Grishan, M. P. 1995. Induction and stability of phenotypic variation in sugarcane as affected by propagation procedure. *Crop Science*, **35**: pp. 875-880.

Chen, W. H.; Davey, M. R.; Power, J. B. and Cocking, E. C. 1998. Control and maintenance of plant regeneration in sugarcane callus culture. *J. Expt. Bot.*, 39: pp. 251-261.

Evans, D. A.; Sharp, W. R. and Medmi-Filho, H. P. 1984. Somaclonal and gametoclonal variation. *American Journal of Botany*, **71**: 759-774.

Hossain, M. A.; Begum, S.; Miah, M. A. S. Uddin, M. J. and Miah, A. J. 1996. Plant Tissue Culture. Oxford and IBH Publishing Co. Pvt. Ltd. New Delhi, pp. 76-79.

Karim, M. Z.; Amin, M. N.; Hossain, M. A.; Islam, S.; Hossain, F. and Alam, R. 2002. Micropropagation of two sugarcane (*Saccharum officinarum*) varieties from callus culture. *On line J.Biological Sci.*, **2**(10): 682-685.

Kochhar, S. L. 1998. Economic Botany in the tropics. Second Edition Macmillan. India Ltd., p. 476.

Mahmud, K.; Nasiruddin, K.M.; Hossain, M.A. and Hossain, L., 2013. *In vitro* regeneration in sugarcane variety Isd 40. *Bangladesh J. Sugarcane*, **33** & **34**: 95-103.

Gopitha, K.; Bhavani, A. L. and Santhilmanickam J. 2010. Effects of the different auxins and citokinins in callus induction, shoot, root regeneration in sugarcane. *International Journal of Pharma and Bio Science*, **1**(3): 1-7.

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

Taylor, P. W.J. 1997. Micropropagation of sugarcane (*Saccharum* spp. Hybrid). *Biotechnology in Agriculture and Forestry*, **39**: pp. 256-268.