Bangladesh J. Sugarcane, ***33 & 34*** : *95-103* *August, 2013*

In Vitro Regeneration in Sugarcane Variety Isd 40

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ABSTRACT

The leaf sheath explants of sugarcane var. Isd 40 were inoculated on MS medium supplemented with varied concentrations of 2,4-D (1.0, 2.0, 3.0, 4.0 and 5.0) mg l^{-1} for callus production and regeneration through this research work. The main effects of MS medium with 2,4-D (1.0, 2.0, 3.0, 4.0 and 5.0) mg l^{-1} and MS medium with Kinetin (0.0, 1.0, 2.0, 3.0, 4.0 and 5.0) mg l^{-1} on callusing, shooting and rooting were investigated. MS medium containing 3.0 mg l^{-1} 2, 4-D produced the highest callus (100 %) among five levels of concentration. The highest number of shoot (8.87) was obtained on MS medium containing 3 mg l^{-1} 2,4-D which statistically similar to MS medium supplemented with 1 mg l^{-1} (8.53) and 4 mg l^{-1} (8.60) 2,4-D respectively. The highest number of roots per shoot (6.60) was found from 1 mg l^{-1} 2,4-D followed by 2 mg l^{-1} 2,4-D (5.60). The highest shoot length (10.73 cm) and root length (2.34 cm) were produced from 2 mg l^{-1} 2,4-D. In case of MS medium containing Kinetin, the highest number of shoot per callus (9.87) was obtained from 2 mg l^{-1} Kn followed by (9.73) 3 mg l^{-1} Kn. On the other hand, the highest number (6.00) of root per shoot was found in MS medium without Kinetin. Besides, MS medium supplemented with 1 mg l^{-1} Kinetin produced the highest shoot length (9.00 cm) and also root length (1.79 cm) respectively. However, the highest number of root per shoot 9.53 and 9.33 were obtained from 1 mg l^{-1} 2,4-D and 0 mg l^{-1} Kn with 5 mg l^{-1} NAA containing MS medium. Therefore, 2,4-D had effect on callus, shoot and root formation while Kinetin had effect on shoot formation in case of sugarcane. Besides, NAA had effect on root formation as well as root increase.

Key words: Sugarcane, Growth regulating hormone 2, 4-D, Callus, shoot and root.

INTRODUCTION

All varieties of sugarcane are species or hybrids of the genus *Saccharum* of the family Gramineae. The genus *Saccharum* contains three cultivated (*Saccharum officinarum*, *Saccharum sinensis* and *Saccharum barbari*) and two wild (*Saccharum robustum* and *Saccharum spontanium*) species (Kochhar, 1998). 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 gur industries. 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). The large barreled high sucrose containing original cane of *Saccharum officinarum* is thought to have originated from the wild species *Saccharum robustum* which is medium thick and low in sugar content (Brandes *et al*, 1939). The

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geographical origin of modern cultivated sugarcane (*Saccharum officinarum*) is New Guinea and distributed throughout the tropics and sub-tropics are generally agreed. Sugarcane is important for sugar, gur and by products such as energy, chemicals, and single cell protein, ethanol, bio-gas, fertilizer, fibre board and paper, polishers, cosmetics and candles. Besides, sugarcane is the greatest source of gur. It is very urgent to increase cane productivity without further area expansion to meet the future need of sugar and gur. The chemical composition of a matured, sound and normal sugarcane stalk of the species *Saccharum officinarum* are water 74.96%, sugar 13.40%, fibre 10.04%, ash 0.64%, N_2 bodies 0.58%, fat and wax 0.38%. Sugarcane is propagated vegetatively for commercial planting by stem cuttings. Production of disease-free large number seedlings during the planting season is laborious and time consuming. Development of more efficient methods for large-scale production of pathogen free planting material would contribute significantly to the overall productivity of the sugar industry. 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). Both average cane yield (45t ha^{-1}) and recovery percentage of sugar (around 8.3 to 8.5%) are fur below as compared to those of the advance sugarcane growing countries. Furthermore, it takes several years of painstaking labour to produce a pure line of sugarcane by selfing due to its high polyploidy and extreme heterozygosity (Narayana Swamy, 1994). Plant tissue culture is the most commercially successful aspect of plant biotechnology, which has introduced an exciting new phase into plant propagation and breeding (Roy Kabir, 2007). Plant regenerated from tissue and cell culture show heritable variation for both qualitative and quantitative trait. Variants are obtained in homozygous condition from the cell in-vitro (R_0 generation, but most variants are recovered in the second generation of the tissue culture regenerated plants (R_1 generation). Somaclonals variation reduces the time for releasing new variety by at least two years as compared to mutation breeding by three years in compared to back cross method of gene transfer. Somaclonal variation had produced desirable agronomic changes in the progeny for example eye spot resistant and increase in sugar yield in sugarcane (Evans *et al.*, 1984). In case of sugarcane, tissue culture induces considerable phenotypic variability (Burner and Grishan, 1995). Callus masses from different explants sources were reported by many scientists like Laurens *et al.* (1987), Chen *et al.* (1998), Shaheen and Mirza *et al.* (1989). Use of different growth regulators in culture medium was reported by Bhansali and Kishan (1982). Many scientists have reported organogenesis and differentiation of shoot from callus culture (Shaheen and Mirza, 1989). Plant tissue culture is considered as a powerful tool for crop improvement within limited time period. For the purpose development of regeneration protocols is prerequisite. In-vitro regeneration of sugarcane has been reported by Barbara and Nickell (1969), Heinz and Mee (1969), Heinz *et al.* (1965) and Barbara *et al.* (1977). 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). Hence tissue culture research using modern sugarcane varieties of Bangladesh deserves due attention. 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 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 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. The sugarcane variety Isd 40 is a fast growing and early-maturing and also well for good quality gur. This variety is tolerant to drought, flood-waterlogging, salinity and also red rot disease. Therefore, this experiment was conducted to find out plantlet regeneration with shooting and rooting potentiality of sugarcane variety Isd 40.

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. The leaf sheath explants were collected from 8-10 months old field grown sugarcane from BSRI experimental field. At first MS medium with green coconut water (10%) containing five concentrations (1, 2, 3, 4 and 5 mg/l) of 2, 4-D was prepared for callus induction. After five weeks of explantation, the calli were inoculated for shooting on MS medium supplemented with different concentrations (0, 1, 2, 3 and 4 mg/l) of Kinetin and maintained by subculturing every two weeks and then regenerated shoots were inoculated for rooting by subculturing every two weeks on MS medium supplemented with 5 mg/l NAA. Rooted plantlets were acclimatized and transplanted to polybag and then field respectively.

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 3% (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 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 and cultured on MS medium with green coconut water containing five concentrations of 2, 4-D (1, 2, 3, 4 and 5 mg/l). Mediums were adjusted to pH (5.7). Agar (0.6%) was added with medium. Media was sterilized by autoclaving at 1.2 Kg cm^{-2} pressure at 121° C for 30 minutes. Cultures were incubated at 25±2°C and kept 16h under fluorescent tube light. The experiment was laid out in Complete Randomized Design (CRD). There 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 Factorial Completely Randomized Design (CRD).

RESULTS AND DISCUSSION

Different concentrations of 2, 4-D had significant effects on callus, shoot, root formation etc in case of leaf sheath explants of sugarcane variety Isd 40 (Figure 1 & Table 1). When explants were inoculated on 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}), the highest callus (100 %) was produced from 2, 4 – D 3 mg l^{-1} followed by 2, 4–D 4 mg l^{-1}(96.7%) and 2, 4-D 2 mg l^{-1}(80.0%). 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) and Gopitha *et al.*, (2010).

The highest number of shoots per callus (8.87) was obtained from MS medium containing 2, 4-D 3 mg l^{-1} followed by 2, 4-D 1 mg l^{-1} (8.53) while the lowest 5.93 from 2, 4-D 5 mg l^{-1}. The highest number of roots per shoot (6.60) was obtained from MS medium containing 2, 4-D 1 mg l^{-1} followed by 2, 4-D 2 mg l^{-1}(5.60). MS medium containing 2, 4-D 2 mg l^{-1} produced the highest shoot (10.73 cm) and root (2.34 cm) length respectively (Table 2).

Different concentrations of kinetin (0, 1, 2, 3, and 4 mg l^{-1}) had significant effects on shoot, root formation etc (Figure 2 & Table 3). When calli were inoculated on MS medium with kinetin (0, 1, 2, 3 and 4 mg l^{-1}), the highest number of shoots per callus (9.87) was found from MS medium containing Kinetin 2 mg l^{-1} and the lowest (5.40) from kinetin 4 mg l^{-1}. The highest number of roots (6.00) per shoot was produced from MS medium without kinetin. On the other hand, the highest shoot (9.40 cm) and root (1.79 cm) length were found from MS medium containing Kinetin 3 and 2 mg l^{-1} respectively (Table 3).

MS medium supplemented with 5 mg l^{-1} NAA derived from different concentration of 2, 4-D and kinetin had significant effects on root (Figure 3 and Table 4). This finding supported the results of Ali *et al.*, (2010); Gill *et al.*, (2002); Singh *et al.*, (2001) and Punia *et al.*, (2001). The highest number of roots per shoot 9.53 and 9.33 were obtained from MS medium containing 5 mg l^{-1} NAA derived from 2, 4-D 1mg l^{-1} and kinetin 0 mg l^{-1}. The regenerated plantlets were transplanted in the polybag and then field after hardening (Figure 4 & 5).

Figure 1: Callus induced from sugarcane variety Isd 40 under concentration of 3 mg/l 2, 4 – D

Figure 2: Regeneration of shoot produced from callus of sugarcane variety Isd 40 after inoculation at 5 mg/l NAA

Figure 3: Regeneration of root produced from callus of sugarcane variety Isd 40 after inoculation at 5 mg/l NAA

Figure 4: Plantlets on the pots

Figure 5: Somaclone in the field

Table 1. Effects of 2, 4-D under different concentration on callus induction.

Treatments	Callus induction (%)
1 mg/l 2, 4-D	76.7 b
2 mg/l 2, 4-D	80.0 b
3 mg/l 2, 4-D	100.0 a
4 mg/l 2, 4-D	96.7 a
5 mg/l 2, 4-D	76.7 b
LSD at 5% level	5.538

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. Main effects of 2, 4-D under different concentration on shoots and roots formation in sugarcane variety Isd 40.

Treatments	Number of shoot/callus	Number of root/shoot	Shoot length (cm)	Root length (cm)
1 mg/l 2, 4-D	8.53 a	6.60 a	10.73 a	1.49 c
2 mg/l 2, 4-D	7.80 b	5.60 b	10.73 a	2.34 a
3 mg/l 2, 4-D	8.87 a	5.33 b	9.20 b	1.37 d
4 mg/l 2, 4-D	8.60 a	4.27 c	7.53 c	1.74 b
5 mg/l 2, 4-D	5.93 c	0.00 d	4.80 d	0.00 e
LSD at 5% level	0.5681	0.3172	0.4865	0.04639

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. Main effects of Kinetin under different concentration on shoots and roots formation in sugarcane variety Isd 40.

Treatments	Number of shoot/callus	Number of root/shoot	Shoot length (cm)	Root length (cm)
0 mg/l Kn	6.53 c	6.00 a	8.87 b	1.77 a
1 mg/l Kn	8.20 b	5.20 b	9.00 ab	1.79 a
2 mg/l Kn	9.87 a	5.00 b	8.87 b	1.48 b
3 mg/l Kn	9.73 a	3.26 c	9.40 a	1.07 c
4 mg/l Kn	5.40 d	2.33 d	6.87 c	0.84 d
LSD at 5% level	0.5681	0.3172	0.4865	0.04639

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 4. Effects of 2, 4-D and Kinetin under different concentration with 5 mg l^{-1} NAA on roots formation/increase of sugarcane variety lsd 40.

2, 4-D mg l^{-1}	Number of root/shoot		Kinetin mg l^{-1}	Number of root/shoot
1	9.53 a		0	9.33 a
2	7.93 b		1	8.87 a
3	8.07 b	and	2	8.00 b
4	8.07 b		3	7.20 c
5	5.67 c		4	5.87 d
LSD at 5% level	0.5289		LSD at 5% level	0.5289

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

ACKNOWLEDGEMENT

We acknowledge to the authority of SPGR-NATP –BARC for supporting to conduct this research work.

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