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Effects of sodium azide on callus in sugarcane

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ABSTRACT

The experiment was conducted at the Biotechnology Laboratory, Bangladesh Sugarcrop Research Institute (BSRI), Ishurdi, Pabna, Bangladesh. The effects of different concentrations of chemical mutagen sodium azide (0, 1, 2, 3, 4 and 5 mg/l for 5 days treatment) on callus of sugarcane varieties Isd 37, Isd 39 and Isd 40 were investigated under in vitro aseptic condition. The highest number of shoots 34.64% was decreased over control (without treated callus) of Isd 39 followed by Isd 37 (25.75%) and Isd 40 (the lowest 21.85%) respectively due to treated by sodium azide (NaN_3). The plantlets regenerations were developed by sodium azide (1-2 mg/l) treated calli derived from MS medium supplemented with 2, 4-D (3 mg/l) containing 10% green coconut water. This is first research work of chemical mutagen sodium azide on callus of sugarcane in Bangladesh.

Key words: Calli, 2, 4-D, NaN_3, Plantlets, Sugarcane and Treated

1. INTRODUCTION

Sugarcane ranks second among cash crops and third among the major field crops in the country and occupies on an average 2.1 percent of the countries total cultivable land (BBS, 2009). The commercial sugarcane, *S. officinarum* has a chromosome number of

2n=80. Modern sugarcane varieties are complex hybrids synthesized from *S. officinarum, S. barberi, S. sinense* and the two wild species, *S. spontaneum* and *S. robustum* (Atikuzzaman, 2008). 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). It is very urgent to increase cane productivity without further area expansion to meet the future need of sugar and gur. 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). 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. Plant tissue culture is considered as a powerful tool for crop improvement within limited time period. Tissue culture of sugarcane has received considerable research attention because of its economic importance as a cash crop. Plant regeneration through tissue culture technique would be a viable alternative for improving the quality and productivity of sugarcane. Tissue culture is widely used in sugarcane improvement. Variety of sugarcane is highly heterogeneous and generally multiplied vegetatively by stem cutting. 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). Due to increased demand of sugar and gur for local consumption, sugarcane is being cultivated years together without adopting modern technologies. Callus culture techniques have been developed as powerful tool for somaclonal variation as well as crop improvement. Natural or induced genetic diversity is a vital component of the crop improvement programme (Patade and Suprasanna, 2008). Sugarcane is vegetatively propagated through setts and work is being carried out on establishing *in vitro* cultures for the purposes of somatic cell genetics through culture-induced mutations and other biotechnological approaches (Suprasanna and Bapat, 2006). *In vitro* culture-induced variability is of common occurrence in tissue cultures (Hoy *et al*., 2003). Therefore, efficient genetic enhancement and propagation system are required for productivity improvement and mass multiplication of sugarcane. Biotechnological approaches such as callus cuture for somaclonal development and micropropagation for rapid multiplication hold great potential for quality production of sugarcane (Sood *et al*., 2006). Chemical mutagens are the one cause of mutations in living organism. It is known that various chemicals have positive or negative effects on living organisms (Fahad and Salim, 2009). There are two types mutagen, chemical mutagens such as Sodium azide (NaN_3), Ethyle Methanesulfonate (EMS), Surflan, Oryzalin, Treflan, Trifluralin, Ethalfluralin, Colchicine, Nitrozo-dimethyl carbamid (NDMC), N-methyl-N'-nitrosoguanodine (NG), N-ethyl-N-nitrosourea (ENU, can be left to natural decay), Methyl methane sulfonate (MMS), Diethylsulfate (DES), Dimethyl sulfoxide, Nitrous acid and physical mutagens such as X-rays, ß-rays, γ-rays, Neutron, UVb (radiation mutagen 280-320 nm). Many researchers compared the mutagenic efficiencies of different mutagens on different crops and their results seem to be entirely specific for particular species and even varieties. As well as many researchers found chemical mutagens to be more effective than physical ones (Dhanayanth and Reddy, 2000; Bhat *et al.*, 2005a). The mutants so produced facilitate the isolation, identification and cloning of genes used in designing crops with improved yield and quality traits (Ahloowalia and Maluszynski, 2001). Sodium azide (NaN_3) is a chemical mutagen and has been one of the most powerful mutagens in crop plants. Chemical mutagenesis (the non-GMO approach) is a simple approach to create mutation in plants for their improvement of potential agronomic traits (Khan *et al.*, 2009). The mutant plants produced by the treatment of sodium azide are capable to survive under various adverse conditions and have improved yields, increased stress tolerance, longer shelf life and reduced agronomic input in comparison to normal plants (Khan *et al.*, 2009). To meet the future requirement of sugar with desirable traits it is essential to develop some improved varieties by callus culture using sodium azide. Hence tissue culture research using sodium azide with modern sugarcane varieties of Bangladesh deserves due attention.

2. MATERIALS AND METHODS

The experiment was conducted at the Biotechnology Laboratory, Bangladesh Sugarcrop Research Institute (BSRI), Ishurdi, Pabna, Bangladesh during the period from 2010 to 2011 to obtain *in vitro* plant regeneration potentiality of BSRI released variety Isd 37, Isd 39 and Isd 40. The leaf sheath explants were collected from 8-10 months old field grown sugarcane from BSRI experimental field. The explants were kept 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 treated with 1% $HgCl_2$ and washed by 3-4 times with sterile distilled water. Explants (approximately 1 cm × 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 3 mg/l. Besides, pH was adjusted to 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. At first MS with green coconut water (10%) containing 3 mg/l of 2, 4-D was prepared for callus induction. Calli were obtained from the best concentration of 2,4–D 3 mg/l and treated with different concentration of mutagen sodium azide (0, 1, 2, 3, 4 & 5 mg/l for 5 days). After treatment, treated calli were transferred into MS including 2,4-D and then transferred into shooting medium containing the BAP (3 mg/l) for proliferation and development of shoots. When small green leaves began to emerge, first sign became evident for successful mutagenic shoot formation as well as regeneration. After inoculation of callus of 20 days on shooting medium, shoots/emerged callus with tiny leaves were aseptically transferred into fresh medium containing the same concentration of BAP for further proliferation and development. Days required for shoot initiation were recorded from inoculation of callus on shooting medium. Data regarding number of shoots per callus, length per shoot, shoot initiation percentage and also increased/decreased shoot (%) were recorded after 40 days of inoculation on first shooting medium. After sufficient amount of shoot formation, the regenerated shoots were aseptically transferred into fresh medium containing NAA (5 mg/l) for proliferation and development of roots. Days required for root initiation were recorded from inoculation of shoot on rooting medium. Data regarding number of roots per shoot, length per root and increased/decreased number of root (%) for mutagen treated were recorded after 40 days of inoculation on rooting medium. Rooted plantlets were transferred in the hardening shade. 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 Completely Randomized Design (CRD).

3. RESULTS AND DISCUSSION

A significant variation was also found due to varieties in respect of shoot and root characters (Table 3.8.4). The least time for shoot initiation (7.61 days) was obtained from Isd 37 while it was maximum (8.50 days) from Isd 39. On the other hand, Isd 40 produced the maximum shoots per callus (4.38) than Isd 37(4.11) and Isd 39 (3.16). On the contrary, Isd 40 noted the shortest time for root initiation (5.94 days) which was statistically similar to Isd 37 (6.11 days) and the maximum roots per shoot (3.50). Furthermore, the maximum requiring time for root initiaon (7.72 days) and the minimum roots per shoot (2.88) were found from Isd 39 and Isd 37, respectively. Effect of different concentration of NaN_3 on various shoot and root characters were significant where there was no shoots and roots were produced by 3.0, 4.0 and 5.0 mg/l NaN_3. Among other concentrations of NaN_3, the shortest time for shoot initiation (10.89 days) was obtained from without NaN_3 while it was the maximum (19.00 days) in 2.0 mg/l NaN_3 treated callus. On the other hand, the maximum shoots per callus (9.88) and roots per shoot (11.33) were obtained from without NaN_3 followed by 1mg/l NaN_3 treated callus (7.22 and 4.33, respectively). The longest time for root initiation (15.11 days) was noted from those shoot which callus was treated by 2.0 mg/l NaN_3 while it was the minimum (8.66 days) from those shoot which was derived from without NaN_3 treated callus (Table 1). This similar finding was supported by Ali *et al.* (2007) who found that the increasing NaN_3 significantly decreased plant regeneration.

Table 1 Main effects of different concentrations of NaN_3 on shoot and root characters at 20 days of inoculation on both shooting and rooting media

Sodium azide (mg/l)	Days to shoots initiation	No. of shoots/callus	Days to roots initiation	No. of roots/shoot
0	10.89 c	9.88 a	8.66 c	11.33 a
1	18.11 b	7.22 b	13.78 b	4.33 b
2	19.00 a	6.22 c	15.11 a	3.77 c
3	-	-	-	-
4	-	-	-	-
5	-	-	-	-
$LSD_{(0.05)}$	0.469	0.391	0.451	0.368
CV (%)	6.13	10.50	7.53	11.88

In a column, figure having same letter(s) do not differ significantly by DMRT at the 5% level

The combined effect between varieties and different concentration of NaN_3 had significant in respect of various shoot and root characters (Table 2). Among the varieties, the least time for shoot initiation (9.66 days) was found from untreated by NaN_3 callus of Isd 37. Similarly, least time for root initaiton (8.33 days) was found from without NaN_3 treated callus of Isd 39 while statistically similar time (8.66 days) was also obtained in without NaN_3 treated callus of Isd 37. The maximum requiring time for shoot (20.00)

and root (17.00 days) initiation were noted in 2.0 mg/l NaN_3 treated callus of Isd 39. On the other hand, without treated callus of Isd 40 and Isd 37 exhibited the maximum shoots per callus (10.67 and 10.33, respectively) while without NaN_3 treated callus of Isd 39 porduced the maximum roots per shoot (12.33) which was statistically differed from other treatment combinations. Similarly, 2.0 mg/l NaN_3 treated callus of Isd 39 produced minimum shoots per callus (4.66) and Isd 37 produced maximum roots per shoot (3.33) (Table 2 and Figure 1).

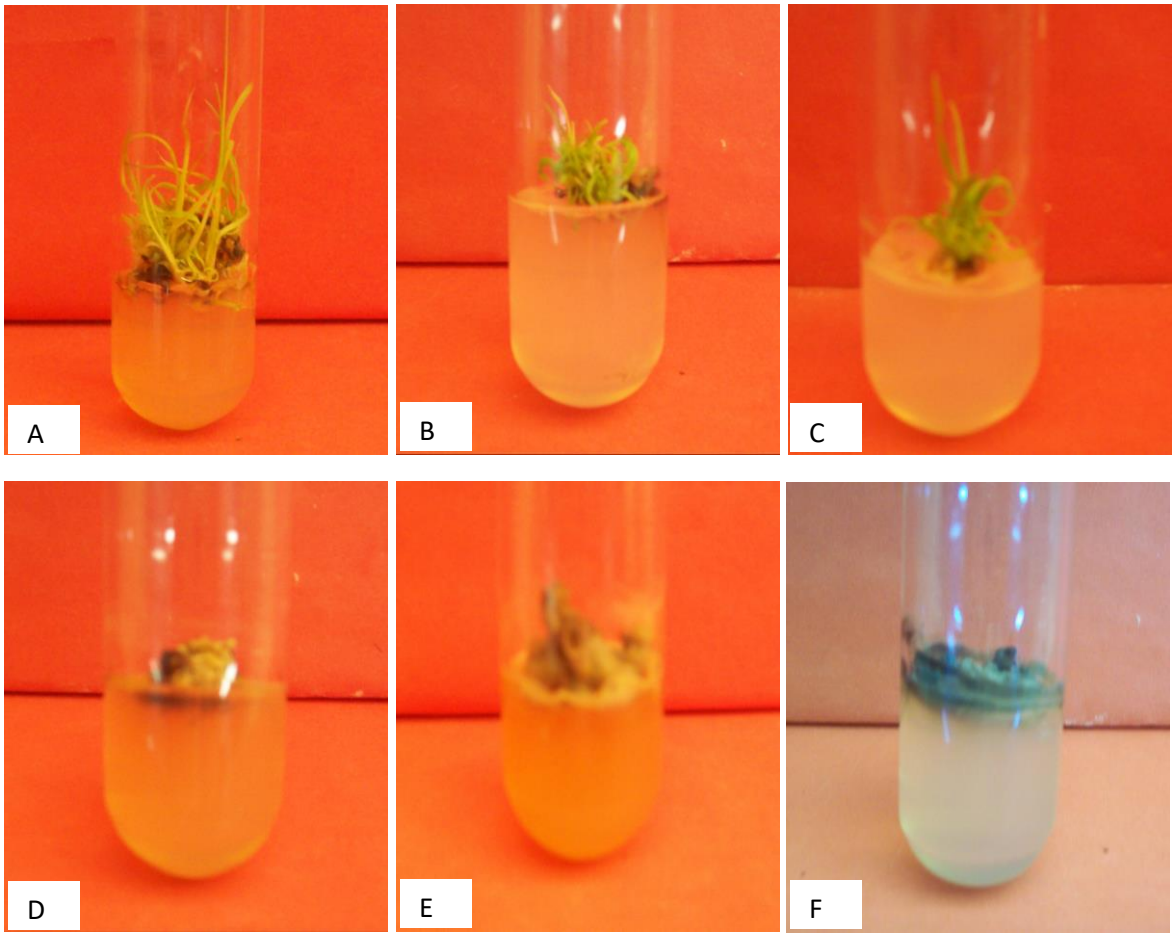

Figure 1

Mutagenic effect of different concentration of sodium azide on callus of sugarcane Isd 37 at 20 days of inoculation on shooting media. A) Shoot produced at 0 mg/l NaN_3 (without treated) B) shoot produced at 1 mg/l NaN_3, C) Shoot produced at 2 mg/l NaN_3 D) No shoot produced at 3 mg/l NaN_3 E) No shoot produced at 4 mg/l NaN_3 F) Callus dead at 5 mg/l NaN_3

Table 2 Combined effects of varieties and NaN_3 concentrations on shoot and root characters at 20 days of inoculation on both shooting and rooting media

Source varieties × NaN_3 concentrations (mg/l)		Days to shoot initiation	Number of shoot per callus	Days to root initiation	Number of root per shoot
Isd 37	0	9.66 f	10.33 a	8.66 d	10.33 c
	1	17.67 c	7.66 cd	13.67 bc	3.66 ef
	2	18.33 bc	6.66 e	14.33 b	3.33 f
	3	-	-	-	-
	4	-	-	-	-
	5	-	-	-	-
Isd 39	0	12.33 d	8.66 b	9.00 d	12.33 a

	1	18.67 b	5.66 f	14.33 b	4.00 ef
	2	20.00 a	4.66 g	17.00 a	3.66 ef
	3	-	-	-	-
	4	-	-	-	-
	5	-	-	-	-
Isd 40	0	10.67 e	10.67 a	8.33 d	11.33 b
	1	18.00 bc	8.33 bc	13.33 c	5.33 d
	2	18.67 b	7.33 de	14.00 bc	4.33 e
	3	-	-	-	-
	4	-	-	-	-
	5	-	-	-	-
$LSD_{(0.05)}$		0.813	0.677	0.780	0.632
CV (%)		6.13	10.5	7.53	11.88

In a column, figure having same letter(s) do not differ significantly by DMRT at the 5% level

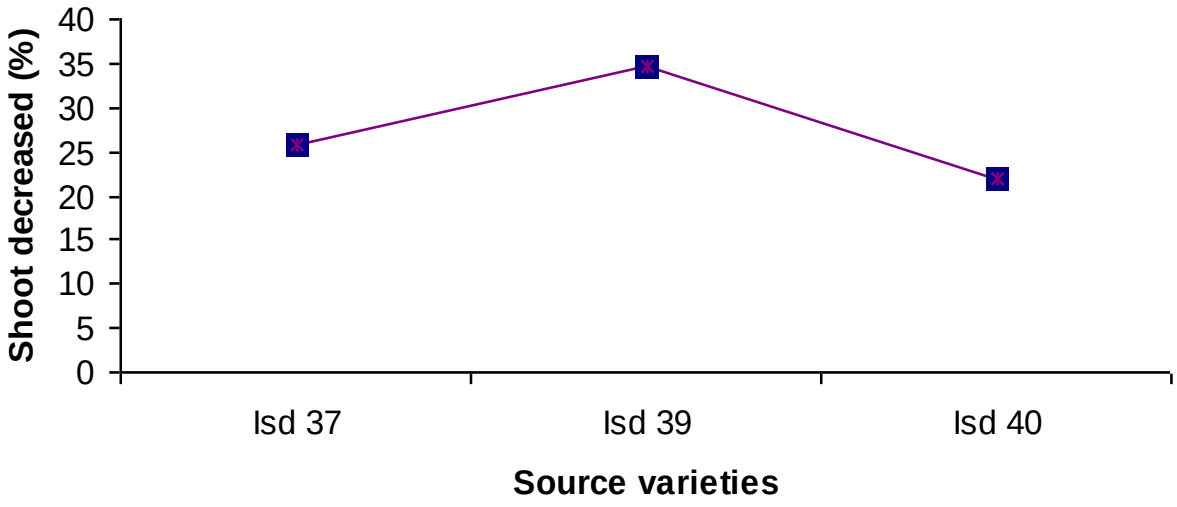

Figure 2

Shoot decreased (%) over control for Sodium azide application at 20 days of inoculation on shooting media

The maximum shoots (34.64%) was decreased over control from callus of Isd 39 source variety than Isd 37 (25.75%) and Isd 40 (21.85%) due to treated by NaN_3 (Figure 2).

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