

Improvement of sugarcane (*Saccharum officinarum* L. var. Isd. 39) using gamma irradiation and large scale plantlet production from M₁ generation through *in vitro* culture

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Abstract

The present study was aimed to develop mutant sugarcane (*Saccharum officinarum* L. var. Isd. 39) using gamma radiation followed by regeneration of those mutants through *in vitro* technique. Five different doses of gamma radiation (10, 20, 30, 40 and 50 Gy) were applied to the sugarcane setts and among these doses 10 Gy irradiated mutant sugarcane showed the best agronomical traits. In addition, 10 Gy irradiated mutant sugarcane also contained higher sugar concentrations (14.25%) compared to control (14.23%). On the other hand, shoot tip and young folded leaf segments from the 10 Gy irradiated plants (M₁ generation) were used as explants in *in vitro* culture. It was evident from the experiment that direct shoot regeneration was better than indirect regeneration. Young leaf segments produced the highest percentage (84%) of shoots when cultured on MS+1.0 mg/l Kin + 0.2 mg/l NAA. The mean number of shoots was recorded as 35 ± 1.12 in the same medium. Moreover, addition of 4% sucrose and 10% coconut water (CW) in the same medium increased the number of shoot up to 45 per culture. Best rooting was obtained from shoots cultured on half-strength of MS fortified with 1.0mg/l NAA and 0.5mg/l IBA. Well-rooted plantlets were transferred to poly bag containing soil and compost (2:1) for hardening. During hardening 70% plantlets survived, which were subsequently transferred to the experimental field.

Key words: *Saccharum officinarum* L. var. Isd. 39, *In vitro* culture, Explants, Regeneration.

INTRODUCTION

Sugarcane is a tall and relatively strong class of perennial grasses that are known to have high sugar content. Commonly known sugarcane species scientific name is *Saccharum officinarum* which belongs to the family Poaceae. It is one of the most important industrial crops in both the tropical and subtropical regions of the world and is the principal raw material for the sugar industry (Khan *et al.*, 2004). The use of gamma radiation to induce mutation is a method that has been applied in plant breeding to increase genetic variations (Brunner, 1995). It has also been used as an effective method, which can greatly induce high mutation numbers and modify physiological characteristics to create new mutants with improved properties (Ahloowalia and Maluszynski, 2001, Bermejo *et al.*, 2011, Predieri, 2001). True genetic changes are desirable in mutation studies. Many fruitful agronomical changes (high cane yield, high sucrose content) were recorded in the treated material (Anonymous, 1953). In addition,

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tissue culture as a tool of advanced plant breeding when coupled with mutation breeding would become an important and valuable tool in the hands of plant breeders to create genetic variability for the selection of new genotypes with improved agronomic characteristics.

On the other hand, micropropagation is currently the only realistic means of achieving rapid, large-scale production of disease-free quality planting material as seed canes of newly developed varieties in order to speed up the breeding and commercialization process in Sugarcane (Feldmann *et al.*, 1994; Lal & Krishna, 1994; Lee, 1987; Lorenzo *et al.*, 2001; Krisnamurthi and Tlaskal, 1974). As a result of which plant regeneration through tissue culture technique would be a viable alternative for improving the quality and productivity in sugarcane.

Sugarcane (*Saccharum officinarum* L. var. Isd. 39) is one of the important agricultural cash crops in Bangladesh and is the major source of sugar. Unfortunately, scientific information about the application of mutation breeding by gamma radiation to improve mutant and from which *in vitro* tissue culture for large scale production is very much scanty or absent. So, the present study was conducted to improve a sugarcane variety (*Saccharum officinarum* L. var. Isd. 39) by gamma irradiation and to establish a protocol for large scale plantlets production of that mutant through *in vitro* culture.

MATERIALS AND METHODS

The investigation was performed in two phases. First phase was gamma radiation treatment in a sugarcane variety for induction of mutation and the second phase was *in vitro* propagation of the superior mutant.

High yielding variety of sugarcane (*Saccharum officinarum* L.) namely Isd. 39 were collected from Bangladesh Sugarcane Research Institute (BSRI), Ishwardi, Pabna. About 900 cane setts containing one eyebud were used as plant material. About 900 cane setts were prepared from collected sugarcane. Thereafter, total cane setts were divided into six batches. First batch was treated as control and rest of the batches like 2, 3, 4, 5 and 6 were subjected to 10, 20, 30, 40 and 50 Gy gamma radiations respectively in a 50,000-curie panoramic ⁶⁰Co source in a radiation chamber of IFRB, AERE. The irradiated cane setts were then transferred to the pre prepared experimental plots. Various agronomical traits namely plant height, nodal diameter and internodal length were recorded after 1.5, 2.5, 4, 5, 7, 10 and 12 months of interval.

In vitro propagation of superior M₁ generation was adopted in order to maintain the characteristic of it. The germinated plants are M₁ progeny as the seed materials were exposed by induced mutagen (gamma ray). Shoot tips and young folded leaves of M₁ progeny (selected elite mother plant) were collected from the experimental field and were used as explants. In the present investigation MS and half strength MS media supplemented with different concentrations and combinations of plant growth regulators (PGRs) were used for the purpose of shoot proliferation and root induction. Surface sterilization of explants (shoot tip and young folded leaves) was carried out by 0.1% HgCl₂ solution for 6 minutes. Thereafter explants cut into small pieces and inoculated in

the culture vessels containing nutrient medium. The culture vessels containing explants were incubated in a growth chamber. The temperature of the growth chamber was $25\pm 2^{\circ}\text{C}$, 16 hours photoperiod and 3000 lux (approx.) photo intensity. Subculture for shoot multiplication and root induction were done at a regular interval of four weeks. The regenerated healthy plantlets after developing sufficient root system were transferred to the soil. The percentage (%) of contamination free explants, the responses of different explants towards shoot regeneration and rooting were recorded carefully. Duncan's multiple-range test (DMRT) was carried out at 5% probability level according to Federer (1963).

RESULTS AND DISCUSSIONS

In the present study, regarding the agronomical traits the percentage of germination was highest (70%) in controlled plants and the second highest percentage was in 10 Gy (48%) irradiated plants among all the irradiated plants. The other irradiated plants i.e., 20, 30 40 and 50Gy showed the rate of germination as 42%, 36%, 30% and 24% respectively. The lowest percentage (24%) was observed in 50 Gy irradiated plants. According to Kwon-Ndung and Ifenkwe (2000), 4 to 8 KGy were identified for optimal germination of cane buds and higher doses decrease the percentage of germination. Higher doses of gamma rays delay and suppress germination of sugarcane buds. Nwachukwu *et al.* (1990) observed similar behavior with sprouting of gamma treated yam micro-tubers. The present results confirm these earlier reports in soybean (Pepol and Pepo 1989 and Pavadai *et al.*, 2009); mung bean and chickpea (Khan *et al.*, 2009 and Khan & Wani, 2005) and sesame (Prabhakar, 1985). Yasmin *et al.* (2011) observed maximum and minimum plant regeneration in 20 Gy and 40 Gy irradiation respectively.

Among all the irradiation, 10 Gy showed best performance in plant height (Table 1). Khan *et al.* (2007) observed that 20 Gy irradiated mutant showed best performance in plant height. This observation was more or less similar with the present findings. In the present study the highest nodal diameter recorded after five, seven, ten and twelve months were 4.1 cm, 5.2 cm, 7.3 cm and 7.5 cm respectively in 10 Gy irradiated plants whereas, the lowest nodal diameter was in 50 Gy irradiated plants (Table 1). Again, the 10 Gy irradiated plants showed the best internodal length among all the plants which were 2.2 cm, 3.5 cm, 8.1 cm and 8.2 cm after five, seven, ten and twelve month of sowing respectively. Plant height and plant girth are the main contributing traits in determining cane yield (Rehman *et al.*, 1992; Khan *et al.*, 1997). In addition, 10 Gy irradiated mutant sugarcane also showed somewhat highest sugar content (14.25%) compare to others (Table 1).

On the other hand, present investigation was undertaken to establish a protocol of *in vitro* plant regeneration from shoot tip and young folded leaf segment explants of improved M₁ line. Total experiment was performed in three phases. In first phase direct regeneration of plants was tried with shoot tip and young folded leaf segments. In second phase, indirect regeneration was tried to establish from young folded leaf segment derived callus. Finally in the third phase rooting, hardening and establishment of plantlets to experimental field was done.

Table 1. Agronomical characteristics of the mutants and controlled sugarcane plants during the study periods

Month	Plant height (cm)						Nodal diameter (cm)						Internodal length (cm)						Sugar content (%)					
	Co nt.	10 Gy	20 Gy	30 Gy	40 Gy	50 Gy	Co nt.	10 Gy	20 Gy	30 Gy	40 Gy	50 Gy	Co nt.	10 Gy	20 Gy	30 Gy	40 Gy	50 Gy	Co nt.	10 Gy	20 Gy	30 Gy	40 Gy	50 Gy
1.5	11.25a	14.06a	13.25a	11.20a	10.06a	9.60a	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2.5	12.75a	21.31b	15.62a	12.75a	11.75a	11.2a	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
4	28b	47.8c	38.87d	31.66c	18a	16.5a	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
5	-	-	-	-	-	-	3.5ab	4.1b	3.6a	3.2a	3.2a	3.1ab	2bc	2.2d	2.1cd	2bc	1.9ab	1.8a	-	-	-	-	-	-
7	16.0cd	16.5d	15.8c	15.0b	14.8b	14.0a	4.8b	5.2b	4.3a	4.2a	4.3a	3.8a	3.1a	3.5b	2.9a	3a	2.8a	2.9a	-	-	-	-	-	-
10	26.1cd	27.0d	25.7c	25.2b	24.7b	24.1a	6.9c	7.3d	6.4b	6.2ab	6.2ab	5.9a	7.3b	8.1c	7.2b	6.8a	6.9a	6.7a	-	-	-	-	-	-
12	36.5bc	37.5c	36.1b	35.5ab	35.2ab	34.5a	7d	7.5e	6.6c	6.3b	6.2ab	6a	7.4b	8.2c	7.3b	6.9a	6.9a	6.8a	14.23	14.25	14.23	14.22	14.22	14.21

*Mean values followed by the same letter in the same column are not significantly different based on DMRT (P=0.05).

Table 2. Effects of different concentrations and combinations of PGRs in MS on shoot regeneration of *Saccharum officinarum* L. var. Isd. 39 using shoot tip and young folded leaf segment from 10 Gy irradiated M₁ generation

Growth regulators (mg l ⁻¹)	Explants			
	Shoot tip		Young folded leaf	
	% of explants producing shoots	Mean number of shoots per culture (±SE)	% of explants producing shoots	Mean number of shoots per culture (±SE)
BAP (mg/l)				
1.2	20c	2±0.09bc	30c	5±0.09c
1.5	26d	3±0.25cd	40c	9±0.25d
2.0	60f	6±1.33e	64d	12±1.35e
2.2	42e	4±1.12d	44c	5 ±1.08c
2.5	28d	3±0.42cd	20c	4 ±0.25c
Kin (mg/l)				
1.0	12b	4 ± 0.08bc	20d	5 ±1.45b
1.2	30d	5 ± 0.09c	28e	9 ±1.34c
1.5	50e	9 ±1.25d	15c	10 ±1.09cd
2.0	68f	13 ±1.32e	70f	12±1.12d
2.2	22c	3±0.24bc	18cd	5±1.08b
3.0	10b	2±0.12ab	8b	5 ±1.13b
Zeatin (mg/l)				
1.0	40d	5 ± 1.12b	30e	15 ± 1.22d
1.2	50e	12 ± 1.45c	20c	8 ± 1.12c
1.5	20c	4 ±0.62b	48f	20 ±3.22e
2.2	48e	10±2.22c	10b	8±1.32c
2.5	60f	16 ±2.12d	8b	8±1.62c
BAP+ NAA (mg/l)				
0.5+ 0.2	30c,c	5 ± 1.18b,b	-	-
1.0+0.3	40d	6 ± 1.14b,c	34d,b	3 ± 1.14c,b
1.0+ 0.5	30d,b	2±1.25b,a	-	-
1.5+0.1	-	-	22c,c	6 ± 1.15d,c
1.5+ 0.2	68e,c	25 ± 2.44c,b	68c,d	20 ± 1.85d,d
1.5+0.3	30e,c	5 ± 1.22c,c	12c,b	4 ± 1.04d,b
2.0+ 0.3	74f,c	30 ± 1.14d,c	08b,b	1 ± 0.08b,b
2.0+ 0.5	52f,b	5±1.25d,a	-	-
Kin+ NAA (mg/l)				
1.0+ 0.1	40d,b	8± 1.12c,b	40e,c	5 ± 1.22d,b
1.0+ 0.2	60d,c	15±1.25c,b	84e,d	35 ± 1.12d,d
1.5+ 0.2	30c,c	3 ± 1.12a,b	20d,d	4 ± 1.12c,d
1.5+ 0.5	35c,c	4±1.08a,b	10d,b	6 ± 1.24c,c
2.0+ 0.1	42e,b	12 ± 1.38d,b	20c,c	5 ± 1.22b,b
2.0+ 0.2	38e,c	10 ± 1.42d,b	-	-
2.0+ 0.5	70e,c	20 ± 1.32d,b	-	-

*- = no response *Mean values followed by the same letter in the same column are not significantly different based on DMRT (P=0.05).

Table 3. Effects of different concentrations and combinations of auxins in half-strength MS on adventitious root formation of *in vitro* raised sugarcane shoots. (Data were recorded after four weeks of culture)

Auxins (mg/l)	% of shoots rooted	Average no. of roots per shoot (\pm SE)	Average length of roots (cm) (\pm SE)
IBA			
0.5	-	-	-
1.0	20b	3 \pm 0.62b	3.0 \pm 0.40b
1.5	30c	4 \pm 0.32a	3.5 \pm 0.70a
2.0	-	-	-
2.5	-	-	-
NAA			
0.5	-	-	-
1.0	-	-	-
1.5	30a	5 \pm 0.56a	4.2 \pm 0.72a
2.0	20c	4 \pm 0.25b	3.4 \pm 0.44c
2.5	-	-	-
IBA + NAA			
0.5 + 0.5	-	-	-
1.0 + 0.5	30c	4 \pm 0.12b	3.0 \pm 0.20b
1.5 + 0.5	50d	7 \pm 0.52c	4.2 \pm 0.32b
2.0 + 0.5	10b	3 \pm 0.43b	3.0 \pm 0.52b
2.5 + 0.5	-	-	-
NAA + IBA			
0.5 + 0.5	-	-	-
1.0 + 0.5	98d	12 \pm 0.36c	3.8 \pm 0.28b
1.5 + 0.5	70c	10 \pm 0.25b	3.4 \pm 0.44b
2.0 + 0.5	50b	8 \pm 0.12b	3.2 \pm 0.32b
2.5 + 0.5	-	-	-

* – = no response. **Mean values followed by the same letter in the same column are not significantly different based on DMRT (p=0.05)

Shoot tip and young folded leaf segments from the 10 Gy irradiated plants (M_1 generation) were cultured onto MS medium supplemented with different concentrations and combinations of cytokinins (BAP, Kin and Zeatin) and auxin (NAA) for direct shoot regeneration. Young leaf segments produced the highest number of shoots when cultured on MS+1.0 mg/l Kin + 0.2 mg/l NAA and about 84% explants proliferated shoots in this particular combination (Table 2). The mean number of shoots per culture was 35 ± 1.12 in the same medium (Plate 3). For indirect regeneration, 1.5 mg/l 2, 4-D with 10% coconut water (CW) induced the highest percentage (96%) of white friable calli from young leaf segments (Plate 1). MS+1.0 mg/l Zeatin + 0.2 mg/l NAA along with 10% CW was found best for indirect shoot regeneration (Plate 2). It was evident from the experiment that direct shoot regeneration was better than indirect regeneration. However, addition of 4% sucrose and 10% coconut water in the medium (MS + 1.0 mg/l Kin + 0.2 mg/l NAA) increased the number of shoot up to 45 per culture (Plate 4). Again, the highest shoot length (7.2cm) was observed when urea (100mg/l) was added to same medium (Plate 5). Finally, MS + 1.0 mg/l Kin + 0.2 mg/l NAA + 4% sucrose + 10% CW + 100mg/l urea was selected for large scale shoot regeneration (Plate 6). On the other hand, Half-strength

MS with 1.0 mg/l NAA and 0.5 mg/l IBA showed best root induction (Plate 7). Percentage of shoots rooted, average number of roots and average lengths of roots were 98, 12 ± 0.36 and 3.8 ± 0.28 respectively on above mentioned medium (Table 3).

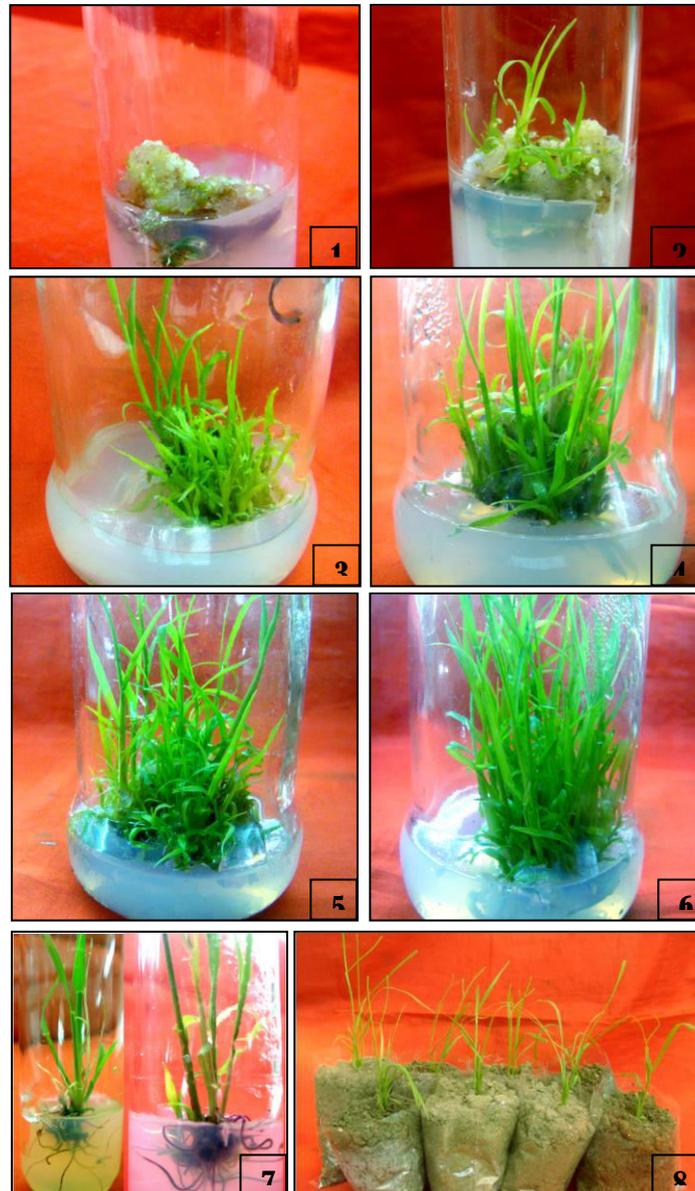


Plate.1-8. *In vitro* regeneration of *Saccharum officinarum* L. var. Isd. 39. 1. Callus induction from folded leaf segment on MS + 1.5 mg/l 2,4-D + 10% CW. 2. Shoot regeneration from callus on MS+ 1.0 mg/l Zeatin + 0.2 mg/l NAA along with 10% CW. 3. Multiple shoots regeneration from young leaf segment on MS+1.0 mg/l Kin + 0.2 mg/l NAA. 4. Positive effect of 4% sucrose and 10% CW on shoot development. 5. Elongated shoots on MS+ 1.0 mg/l Kin + 0.2 mg/l NAA+100mg/l urea. 6. Healthy and elongated multiple shoots on MS + 1.0 mg/l Kin + 0.2 mg/l NAA + 4% sucrose + 10% CW + 100 mg/l urea. 7. Root induction on half -strength MS + 1.0 mg/l NAA + 0.5 mg/l IBA. 8 Acclimatized plantlets in poly bag soil

There are many reports on regeneration of shoots from shoot tip explant of mature plants. Pathak *et al.* (2009), Singh *et al.* (2001) reported that optimum number of shoot was produced in MS medium supplemented with BAP, Kin and NAA (0.5 mg/l each). Similar results were obtained by Biradar *et al.* (2009) with BAP (2.0 mg/l), Dhumale *et al.* (1994) with BAP at the rate of 3.0 mg/l and NAA with 1 mg/l in case of sugarcane which supported the present findings. Ali *et al.* (2008) observed the best shoot regeneration from shoot tip explants in MS medium with 1.5 mg/l BAP. Roy and Kabir (2007) reported best shoot proliferation in MS media with 1.5 mg/l BA from shoot tip explants. For acclimatization and plant establishment under the natural conditions, the well-rooted plantlets were transferred to poly bag containing soil and compost in the ratio of 2:1 (Plate 8). During hardening 70% plantlets survived and these were subsequently transferred to experimental field.

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