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In vitro Regeneration of Sugarcane (*Saccharum officinarum* L) through Gamma Irradiation

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Abstract

The present research work was carried out employing gamma irradiation to create mutant Sugarcane (*Saccharum officinarum* L.) and in vitro regeneration to regenerate those mutants. Sugarcane buds were exposed to three doses of gamma radiation (20, 30 and 40Gy) using 60Co and the agronomical properties of 20 Gy irradiated mutant sugarcane were the best among these dosages. Furthermore, 20 Gy irradiated mutant sugarcane had higher sugar contents (22%) than control sugarcane (18%). In contrast, standardizing of callus induction and plantlet regeneration protocols from 20 Gy irradiated sugarcane plants (M1 generation) was established through in vitro culture using young meristem as an explant. Using various concentrations and combinations of growth regulators, shoot regeneration at varying frequency was recorded. In MS media enriched with 2.5 mg/l, 2-4 D, callus induction was detected. On MS medium with BAP 2.0 mg/L + NAA 0.5 mg/L, the best response in terms of multiple shoot induction was reported. Rooting was more profuse when in vitro shootlets were placed on half-strength MS basal media supplemented with 2.0 mg/L NAA. Hardening shoots were transplanted into the green house after they had been rooted.

Keywords: *Saccharum officinarum* L., Callus Induction, Shoot Multiplication, Root Formation, 2-4D, BAP, NAA.

INTRODUCTION

Sugarcane (*Saccharum officinarum L.*) is an important agricultural cash crop in tropical and subtropical region of the world and is the major source of sugar with respect to export product in many developing countries that accounts for more than 60% of the world's sugar production [1]. In 2020, the world's total sugar cane harvested area was predicted to be 26.4 million ha. Most of the world's sugar is produced from sugarcane and in recent years, that's meant a total of about 1.8 billion tons of harvested sugarcane annually. There is a great need to start breeding of high yielding cultivars of sugarcane. However, Conventional breeding research approaches had already been explored in the past to improve genetic base of sugarcane for solving the constraints limiting productivity. Mutation breeding has been widely employed to improve plant characteristics in a variety of crops. In the hands of plant breeders, it is a widely used and effective tool.

It is therefore imperative that technological interventions that circumvent the problems associated with the conventional propagation methods are found and implemented to address the problem of low sugarcane productivity in Myanmar. There is much evidence to prove the usefulness of in vitro culture combined with induced mutation for bringing about the desirable characters of high yield in the crop plants [2-4].

In vitro techniques provide the mechanism to generate large population for mutation induction and rapid multiplication of the selected mutants [5,6]. The availability of large population for mutagenesis is one of the basic pre-requisites to obtain sufficient variation. This study investigated the effects of different doses of gamma radiation on sugarcane and explored their association with the stem strength and yield-contributing parameters and produce large number of sugarcane plants through in vitro regeneration techniques.

MATERIALS AND METHODS

Plant material and conducted Gamma irradiation

The present study was conducted at Department of Biotechnology Research (DBR). Laboratory experiments were conducted to standardize a protocol for large scale and low-cost production of meristem cultured (tissue culture) plants of sugarcane through Gamma radiation. During this study, sugarcane (*Saccharum officinarum L.)* variety were irradiated with three different dose of gamma rays (20 Gy, 30 Gy and 40 Gy). Irradiated sugarcane segments were planted to the field for purpose of agronomic character study. Field experiments were conducted on the farms of Nyaung Shwe. According to the result of agronomic character in the field, 20Gy was the appropriate dose of the gamma irradiation for induction of variation in sugarcane shoot culture through *in vitro* techniques.

Source of explants and surface sterilization

Meristems are plant growth centers found at the terminals of budding shoots and leaves. Sugarcane meristems and outer cover were rinsed with distilled water, then surface sterilized with 0.2% mercuric chloride for 10 minutes before being washed three times with sterile distilled water to remove chemical traces. With the use of surgical blades, the leaf sheaths covering the meristem were removed aseptically, and meristem explants were recovered. After that, the explants were inoculated by dissecting and sizing the meristem (0.5–1.0 cm) properly.

Culture media and condition

Murashige T [7], in laboratory research to create a methodology for sugarcane meristem cultivation, medium was utilized as a basal media. 3 % sucrose was added to the MS medium. This medium was supplemented with different combinations of cytokinins and auxin; pH of the medium was maintained at 5.8. The culture tubes/- bottles were incubated on illuminated racks at 16 h light and 8 h dark period in 24 h at the room temperature of 25 ℃ in a growth chamber.

In vitro shoot multiplication

Different combinations of cytokinins and auxins were tested to select the best media for shoot formation and root formation in meristem culture of sugarcane. MS media supplemented with different concentration (0, 0.5, 1.0, 1.5 and 2 mg/l) of "6- Benzyl aminopurine" (BAP) on ten explants, shoot multiplication was examined. Each experiment was repeated 3 times to confirm the results. Subculture was carried out every 2–3 weeks.

In vitro rooting of plantlets

Shoots obtained from the above culture bottles were transferred to MS basal medium with 0.5mg/L NAA and for root production, use 1/2 strength MS medium with 0.5 mg/l NAA. Each experiment was repeated 3 times.

Data analysis

Data of shooting and rooting were presented as mean and standard error. Data were analyzed by SPSS software.

RESULTS

In the present study, we observed two portions: the first part focused into using gamma radiation to induce mutations in a sugarcane variety, while the second part examined in vitro propagation of the superior mutant.

In terms of agronomical features, controlled plants had the highest percentage of germination (90%) and 20 Gy irradiated plants had the second highest percentage (78%) among all irradiated plants. The other irradiation plants, which were given 30 and 40 Gy, had a germination rate of 67 and 45 percent, respectively (Figure 1). The lowest percentage (45 %) was observed in 40 Gy irradiated plants. The 20 Gy irradiated mutant had the best plant height performance of all the irradiated mutants (Table 1). The maximum nodal diameters measured after twelve months in irradiated plants were 4.0 cm, 3.5 cm, and 3.1 cm, respectively, whereas the lowest nodal diameter (2 cm) was found in non-irradiated plants (control) (Table 1). Among all the plants, the 20 Gy irradiated plants had the best inter-nodal length (6.5 cm). Moreover, when compared to other treatments and controls, 20 Gy irradiation mutant sugarcane had the highest sugar content (22 %) (Table 1).

On the other hand, the current study aimed to develop a protocol for in vitro plant regeneration using sugarcane meristem explants from the enhanced M1 line. Firstly, callus induction was tested on MS basal medium with 2.5mg/L2,4D. Various combinations and quantities of growth regulators were used to create multiple shoots and roots. The effect of the different combinations of BAP on shoot multiplication and the effect of auxins (NAA) on rooting was tested. Result of this experiment showed that MS medium supplemented with 2.5mg/L 2,4-D showed that good response of callus induction, whereas the maximum in vitro shoot multiplication of sugarcane (61.02 ± 0.904^{a}) was obtained in BAP 2 mg/L combination with NAA0.5 mg/L (Table 2). Among different concentrations and combinations for shoot multiplication, minimum shoot multiplication (37.48 ± 1.10^{d}) was observed in BAP 0.5 mg/l + NAA 0.5 mg/L with mean shoot length (6.26±0.25 cm) follow by BAP 1 mg/L+NAA0.5mg/L (44 ± 0.707^{c}) and BAP1 .5mg/L+NAA 0.5mg/L (49.3 ± 0.80^{b}). MS_0 medium showed that a few numbers of shoot formation however the mean shoot length (8.82 ± 0.89^{a}) is better than other treatments ideal medium (plate 1) and ½ MS supplement with NAA 2 mg/L found that more profuse and longer root formation (plate 2).

Table 1: Agronomical features of mutants and controlled sugarcane plants during the experimental periods (12 months)

Radiation dose (Gy)	Plant Height (Feet)	No. of Nodal	Length of Internodal (inch)	Nodal diameter (inch)	Sugar content (%)	Yield (ton/acra)
20	12	24	6.5	4	22	30
30	11	23	6	3.5	20	28
40	10.5	21	4.7	2.9	19.2	25.9
Control	8	20	3.5	2	18	25

Table 2: The effects of various cytokinin (BAP) concentrations and auxin combinations (NAA) in MS media on callus tissue for shoot regeneration. (Data were recorded after four weeks of culture)

Treatments	Hormone concentration (mg/l)	No. of shoots/ bottle	Average shoot length (cm)
T1	BAP 2 + NAA 0.5	61.02 ± 0.904^{a}	4.44 ± 0.18^{c}
T2	BAP 1.5 + NAA 0.5	49.3 ± 0.80^{b}	4.72 ± 0.33^{c}
T3	BAP 1 + NAA 0.5	44 ± 0.707^{c}	5.14 ± 0.21^{b}
T4	BAP 0.5 + NAA 0.5	37.48 ± 1.10^{d}	6.26 ± 0.25^{b}
T5	MS0 (control)	9.6 ± 0.69^{e}	8.82 ± 0.89^{a}

BAP = 6-Benzyal amino purine: NAA = α-naphthalene acetic acid

[10 duplicates per treatment; twice repeated.] Duncan's multiple range tests are used to calculate the means at a significance level of 5%.]

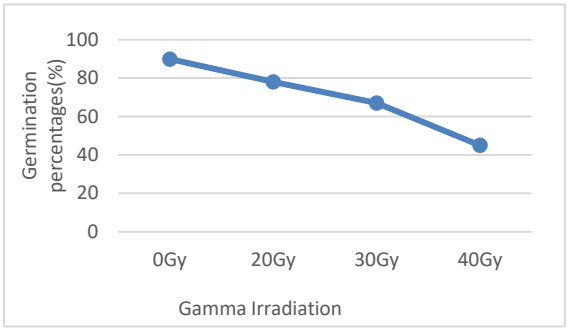

Figure 1: Gamma ray effects on sugarcane germination

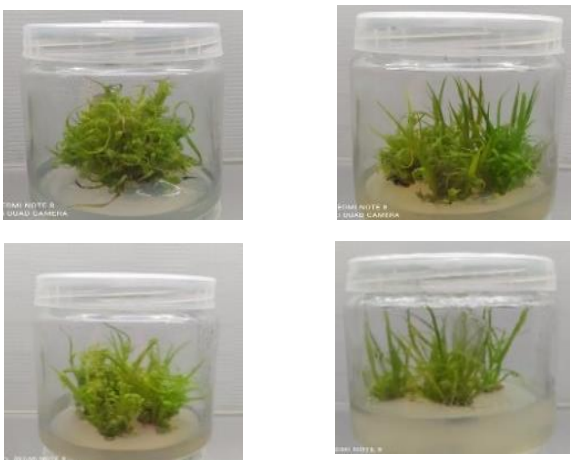

Plate 1: *In vitro* regeneration of *Saccharum officinarum L.* (1) Multiple shoots regeneration from young leaf segment on MS+2.0 mg/l BAP + 0.5mg/l NAA. (2) MS+1.5 mg/l BAP + 0.5mg/l NAA. (3) MS+ 1 mg/l BAP + 0.5mg/l NAA. (4) MS+ 0.5 mg/l BAP + 0.5 mg/l NAA

Plate 2: Micro shoots rooted in 1/2MS + NAA (2.0mg/l)

DISCUSSIONS

The 20 Gy irradiation had the best results in terms of plant height of all the irradiations (Table 1). Khan IA [8] also discovered that the 20 Gy irradiated mutant had the best plant height performance. So that, this observation corresponded to the current findings. The largest nodal diameter measured after twelve months in 20 Gy irradiated plants was 4 cm, while the lowest nodal diameter was measured in 40 Gy irradiated plants (Table 1). Moreover, after twelve months of seeding, the 20 Gy irradiated plants had the best internodal length of all the plants, measuring 6.5 cm, 6 cm, and 4.7 cm after 20 Gy, 30 Gy, and 40 Gy, respectively. The key contributing variables in determining cane yield are plant height plant girth [9,10]. In addition, the current findings support previous findings in soybean [11,12]; mung bean; and chickpea [11]. Khan IA [13], Khan S [14] and sesame seeds [15], in 20 Gy and 40 Gy irradiation, Yasmin S [16] reported maximum and minimal plant regeneration.

For shoot regeneration, different concentrations of cytokinin (BAP) and auxins (NAA) were utilized in different concentrations and combinations. The concentrations and types of growth regulators used in this study had a significant impact on the development of shoots. The highest performance was seen on MS medium supplemented with BAP (2.0 mg/l) + NAA (0.5 mg/l) for shoot multiplication among various concentrations and combinations (Table-2). Explants developed shoots in 92 % as a result by using this combination. Although, this treatment was given the highest shoot number 61.02±0.904, the average length of the shoots 4.44±0.18cm which was the shortest shoot length among other treatments (Table 2.) The MS medium supplemented with BAP (1.5mg/l) + NAA (0.5mg/l) produced the second-best results, with an average number of useable shoots of 49.3±0.80 and a mean length of 4.72±0.33 cm (Table-2). Islam AS [17] also found that combining BAP and NAA had a favorable effect on sugarcane shoot formation. Individual concentrations of cytokinin were found to be less important than combinations of high cytokinin and low auxin for the differentiation of adventitious shoots in sugar cane early meristem callus. All of these investigations found that callus regeneration was a genotype-dependent phenomenon that paralleled hormone concentrations and combinations [18]. Sugarcane callus induction, proliferation, and regeneration potential were all synchronized [19].

CONCLUSION

Plant tissue culture could be a significant component of the "Second Green Revolution," in which biotechnology and gene modification are utilized to boost crop productivity and quality. The present study was concluded that 20 Gy irradiated sugarcane was potential used for improvement of plant agronomical quality and combinations of BAP and NAA were suitable for plant regeneration of sugarcane in future research.

Conflict of Interest

None declared.

Financial Support

None declared.

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