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In vitro Regeneration of Sugarcane (*Saccharum officinarum*) Varieties GUI 11 and PMA 96/48

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Abstract

The present investigation is deals with in vitro regeneration of two sugarcane varieties from Yezin, Pyinmana, Myanmar. The young suckers of two sugarcane varieties GUI11 and PMA 96/48 were used as explants. In this research, both sugarcane varieties gave high shoot proliferation rate on MS medium plus hormone BAP and NAA combinations. The shoot regeneration results of both varieties were not significant on MS basal medium supplemented with BAP + IBA (1+0.2, 1+0.5, 1+0.7, 0.5+0.1, 0.5+0.2, 0.5+ 0.3 mg/l). The maximum shoot formation was observed in MS medium supplemented with BAP 1mg/l + IBA 0.2 mg/l for both GUI 11 and PMA 96/48. The rooting experiment was conducted in half strength MS medium with various concentrations of NAA. The root formation from regenerated shoot was observed in half strength of MS medium supplemented with NAA 4, 5, 6 mg/l for both sugarcane varieties. Among three levels of NAA tested, the highest root formation was found at 5mg/l in GUI 11 and 6mg/l in PMA 96/48.

Keywords: *In vitro* regeneration, BAP, NAA, Sugarcane, Myanmar, MS medium.

INTRODUCTION

Sugarcane is a member of the family Graminae with the genus *Saccharum* between 6-37 species (depending on taxonomic interpretation) and it is native to warm temperate to tropical region of the old world. Commercial sugarcane cultivars are developed by crossing the domested species *Saccharum officinarum* L. (2n = 80) and the wild species *Saccharum spontaneum* (2n= 40-120) and so they are interspecific poly-aneuploid hybrids with chromosome numbers in excess of 100 [1].

Sugarcane is originally grown in the South Pacific islands and New Guinea (Irvine 1981) [2]. Nowadays, Brazil is a major grower of sugarcane crops where it is used to produce sugar as well as to provide the alcohol subsequently used in making gasohol and biodiesel fuel [3] In Myanmar, sugarcane is one of the most important crops and grown in five Divisions and five States of the country. The total area under sugarcane growing was 67,657,752 hectares in 2001-2002 and national average yield was 44382 kg/hectare (Agricultural Atlas of the Union of Myanmar, Agricultural Year 2001-2002) [4]. The problem in sugarcane multiplication has long been due to lack of suitable multiplication procedure in sugarcane breeding programme in many years ago [5]. Tissue culture technology presents an opportunity for the rapid multiplication of sugarcane. Using micropropagation technique, over 1.5 million sugarcane plantlets can be produced from a single shoot tip explant in six months of the culture period [6]. Therefore, plant regeneration using plant tissue culture technique would be a viable method for improving the quality and production of sugarcane [7].

The present study has been aimed at followings; to recognize the regeneration potential of two sugarcane varieties GUI 11 and PMA 96/48 in supplementation of various auxin and cytokinin ratio on MS (Murashige and Skoog 1962) [8].

MATERIALS AND METHODS

a) Source of Explants

The two sugarcane varieties GUI 11 and PMA 96/48 obtained from Myanma Sugarcane Enterprise, Pyinmana, Myanmar. The top portions of the 3 to 6 month-old field-grown sugarcane plants were used as the source of explant material.

b) Explant Collection

The healthy young sugarcane plants with at least 4 to 5 leaves stage were used in present work. They were collected from the plantation of field-grown sugarcane and then the collected materials were washed thoroughly under running tap water. After that the young plants were rubbed with ethanol and wrapped in paper towel. And then, they were brought to the laboratory.

c) Sterilization of Glasswares and Instruments

Firstly, all the glass bottles and instruments used for sugarcane tissue culture were washed with detergent, rinsed in running water and then dry in sunlight.

Glass bottles, petri dishes and instruments like scalpel, forceps and blade handles were wrapped in paper and finally covered with plastic bags. After this preparation, these items were sterilized in autoclave at 121ºC for 30 minutes.

d) Sterilization of Explants

The plants were surface-sterilized with 20% clorox (commercial bleach) for 20 minutes, after which they were washed 3 to 4 times with sterilized double distilled water.

e) Preparation of Stock Solutions

MS Stock Preparation

The stock solutions were composed to form MS medium as A, B, C, D, E, F and G (10 x concentrations).

A stock solution of 10x was prepared and only a required amount was taken to prepare different treatments. After which sugar was added and media were adjusted to pH 5.8 with drop-by-drop additions of 1N NaCl or 1NHCl using a pH meter. Agar of related amount was added to solidify the media and then the media were poured into glass bottles with the capacity of 100 ml. Each bottle containing 20ml of medium was covered with a sheet of clear plastic and sterilized in an autoclave for 15 min at 121ºC.

f) In Vitro Regeneration for Sugarcane Varieties (GUI 11 and PMA 96/48)

It included callus induction, callus proliferation, shoot multiplication and root formation steps.

In vitro plant tissue culturing, culture bottles were maintained in a growth room at 25±2ºC under constant illumination of 4 feet white fluorescence tube with yield on intensity of 200 Lux during 24 hours photoperiods.

i) Callus Induction

For callus induction, the outer old leaf-base coverings of the surface sterilized plant materials were carefully removed without damaging the inner young and delicate tissues. After removing the outer sheaths, the innermost three whorls of leaf portions were sliced into 3-4 mm long pieces. The aseptically excised plants were inoculated on MS medium supplemented with 3mg/l 2,4-D in bottles each containing 20ml of medium. The medium for callus induction was MS basal medium supplemented with 6 g/l agar, 3mg/l 2,4-D and 20 g/l sucrose. The induced calli were maintained or proliferated on the same medium by subculturing after every 2-3 weeks. After about one month, the proembryogenic calli were transferred to shoot induction media.

ii) Shoot formation and Shoot proliferation

The proembryogenic calli formed were incubated in modified MS media supplemented with 6g/l agar, 20g/l sucrose and different concentrations and combinations of BAP and IBA (1mg/l+0.2mg/l, 1mg/l+0.5mg/l, 1mg/l+0.7mg/l, 0.5 mg/l+0.1mg/l, 0.5mg/l+0.2mg/l, 0.5mg/l+0.3mg/l respectively). After two weeks, the shoots were formed from the proembryogenic calli of both varieties.

iii) Root Formation

The regenerated sugarcane shoots were transplanted to the rooting media. The root formation medium was half-strength MS medium with 5.5 g/l agar, 50g/l sucrose and different concentrations of NAA (4mg/l, 5mg/l, 6mg/l).Within two weeks of transferring to rooting medium, roots initiated from the bottom of well-grown shoots of two varieties.

g) Culture Condition

The culture bottles for callus induction and root formation were incubated at 27˚±1˚C under16/8hrs light/dark photoperiod and the culture bottles for shoot formation were kept at 27˚±1˚C under continuous fluorescent tube light.

h) Data Analysis and Presentation

For the first experiment, six treatments were tested for shoot proliferation from GUI11 and PMA 96/48. Each treatment for GUI 11 had four replicates and each for PMA 96/48 had six replicates. For the rooting experiment, the replicates for each of three treatments for both varieties were ten. Then these of all experiments were subjected to analysis of variance using IRRISTAT. For presentation, Microsoft Excel was used to prepare the scientific graph.

RESULTS AND DISCUSSIONS

a) Results

i) Effect of different concentrations of 2, 4-D on Callus Induction on two sugarcane varieties GUI 11 and PMA 96/48

Callus initiation started on MS medium supplemented with 2, 4- D (3mg/l) 9 days after inoculation in PMA 96/48 and 11 days in GUI11. Both varieties showed high percentages of callus induction greater than 80%. Based on their morphological appearances, callus types of two cultivars was slightly different; callus type of GUI 11was whitish globular, non-compact and wet and that of PMA 96/48 was yellowish white, compact, slightly dry and globular. The callus induction rate is a bit slower in PMA 96/48 than in GUI 11. The calli of both cultivars which were creamy white color were maintained by subculturing on fresh medium every third week. It has been observed that callus of both varieties initiated from cut edges of shoot tip explants and developed into a full-grown callus within 30 days of culture (Plate 1)

ii) Effect of Different Concentrations and Combinations of BAP and NAA Hormone for Shoot Proliferation

In this experiment, the combination of growth regulators BAP as cytokinin and NAA as auxin (1+0.2,

1+0.5, 1+0.7, 0.5+0.1, 0.5+0.2, 0.5+0.3 mg/l) were used with basal MS medium. Within two weeks, shoot initiated on the calli of both GUI 11

and PMA 96/48. Among different concentrations and combinations of BAP and IBA used for shoot multiplication and elongation, BAP1mg/l+ IBA0.5mg/l was the best performance in GUI 11. In PMA 96/48, the maximum shoot formation was in a combination of BAP0.5 mg/l+ IBA0.2mg/l and the longest shoot length was occurred in BAP 1mg/l + IBA 0.3mg/l. The effects of BAP+IBA in six combinations on growth and development of shoot in GUI 11 and PMA96/48 are shown in Plate 2 and Plate 3. In the formation and elongation of shoots in all media compositions, PMA 96/48 showed better results than the GUI 11 variety.

iii) Effect of Different Concentrations of NAA on root Formation

Healthy and well established shoots from *in vitro* proliferating culture were placed on half strength MS medium supplemented with three different concentrations of NAA viz., 4, 5, 6mg/l. Ten days after transferring to rooting medium, roots of both varieties initiated in almost all different NAA concentrations. Among different concentrations of NAA used, NAA 5 was found to be comparatively better response for producing roots than other two treatments in GUI 11. In PMA 96/48, the best rooting was observed in half strength MS medium supplemented with 6mg/l NAA. Micro-shoots of variety PMA96/48 showed better rooting performance than that of the variety GUI 11 in the same media composition used (Plate 4 and Plate 5).

Table 1: Effects of 3mg/l 2,4-D on the Embryogenic Callus Formation in GUI11and PMA 96/48

Variety	% of Embryogenic Calli	Type and Color of Calli
GUI11	95.238 %	Creamy white, globular, non-compact and wet
PMA 96/48	87.301%	Yellowish white, compact, globular and slightly dry

Data is based on five replicates.

Table 2: Effects of BAP and IBA on the Shoot Formation and Shoot Length of GUI11 after Two Months of Culture.

Concentration (mg/l)	Number of Shoot	Shoot length (cm)
BAP 1+IBA 0.2	142	22
BAP 1+IBA 0..5	177	37.5
BAP 1+IBA 0..7	83	17
BAP 0.5+IBA 0.1	137	26
BAP 0.5+IBA 0.2	109	21
BAP 0.5+IBA 0.3	85	18
Mean	30.542	5.896
CV %	45.7	61.6
F	2.15	3.52
F5%	2.77	2.77
F1%	4.25	4.25

Table 3: Effects of BAP and IBA on the Shoot Formation and Shoot Length of PMA 96/48 after Two Months of Culture.

Concentration (mg/l)	Number of Shoot	Shoot length (cm)
BAP 1+IBA 0.2	202	64
BAP 1+IBA 0..5	245	62
BAP 1+IBA 0..7	237	63
BAP 0.5+IBA 0.1	195	45
BAP 0.5+IBA 0.2	253	68
BAP 0.5+IBA 0.3	221	40.5
Mean	37.583	9.514
CV %	37.9	45.4
F	6.42	1.188
F5%	2.3	2.53
F1%	3.7	3.7

Table 4: Effects of Different Concentration of NAA on Root Formation of GUI11 and PMA 96/48 Two Months after Transferring to Root Formation Medium.

Concentration (mg/l)	GUI 11 Number of Root	PMA 96/48 Number of Root
NAA 4	242	231
NAA 5	305	276
NAA 6	235	385
Mean	26.067	29.733
CV%	29.4	28.4
F	13.5	13.875
F5%	3.35	3.35
F1%	5.49	5.49

Plate 1: Callus Formation on Shoot Tip Culture of GUI11 and PMA 96/48 on MS Medium Supplemented with 2,4-D 3mg/l after One Month of Culture.

Plate 2: Regeneration of Shoot *in vitro* from Leaf Sheath explant of GUI11onMS Medium Supplemented With BAP+IBA (1+0.2,1+0.5,1+0.7,0.5+0.1,0.5+0.2 and 0.5+0.3 mg/l).

Plate 3: Regeneration of Shoot *in vitro* from Leaf Sheath explant of PMA 96/48 on MS Medium Supplemented With BAP+IBA (1+0.2,1+0.5, 1+0.7,0.5+0.1,0.5+0.2 and 0.5+0.3 mg/l).

Plate 4: Root Formation of GUI11on MS Medium with Different Concentrations of NAA (4, 5, 6mg/l).

Plate 5: Root Formation of PMA96/48 on MS Medium with Different Concentrations of NAA (4, 5, 6mg/l).

B. Discussions

In the first experiment reported here, MS medium supplemented with 3mg/l of 2,4-D gave callus formation during 9 days after inoculation in PMA 96/48 and 11 days in GUI11. So, this concentration of 2,4-D is suitable for callus induction in sugarcane.

In the second experiment, different concentrations and combinations of BAP and IBA (1+0.2, 1+0.5, 1+0.7, 0.5+0.1, 0.5+0.2, 0.5+0.3mg/l) were used in MS medium for multiple shoot regeneration from shoot-tip explants of two sugarcane varieties. There was not highly significant among the effects of BAP and IBA on the number of shoot and shoot length of GUI 11 according to the statistical analysis. There was significant difference in shooting responses which was detected among all the six concentrations tested but was not significant difference in shoot length in PMA 96/48.

In the third experiment, the effects of the presence of three concentrations of NAA (4, 5, 6mg/l) were examined during the rooting stage. At three concentrations, NAA 5 mg/l was more favorable for root formation than the NAA4mg/l and NAA6mg/l in GUI11. On the other hand, at those concentrations, NAA6 mg/l was the best rooting in PMA 96/48. According to data analysis, there was greatly significant difference among three treatments in both two varieties.

CONCLUSIONS

In callus formation, MS medium supplemented with 3mg/l 2,4-D showed callus formation in both varieties, but the culture response of GUI 11 was better than that of PMA 96/48. Therefore, this hormone concentration is very useful for callus formation of GUI 11 and PMA96/48. In shoot formation, it was observed that the best shooting for GUI 11 was MS basal medium supplemented with BAP 1mg/l + IBA 0.5mg/l and that for PMA 96/48 was MS medium supplemented with BAP0.5+IBA0.2mg/l. Thus, it can be concluded that BAP combined with IBA in modified MS medium promoted shoot regeneration from embryogenic callus in this sugarcane varieties. For root formation, using MS medium supplemented with different concentrations of NAA (4, 5, 6mg/l), the highest rooting was observed in NAA 5 mg/l in variety GUI 11 and the highest of which was in NAA 6 in variety PMA 96/48. In this experiment, the multiple root formation was observed in both varieties, therefore it is suitable for rooting.

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REFERENCES

1. Heinz, *et al. Cell, Tissue and Organ Culture in Sugarcane Improvement. An Applied and fundamental aspecies of plant cell, tissue and organ culture.* Berlin, Heidelburg, New York, 1977, pp. 3-17.
2. Irvine, *et al. The frequency of marker changes in plants regenerated from callus culture. II. Evidence for vegetative and genetic transmission, epigenetic effects and chemical disruption.* Plant Cell, Tissue.
3. Liu MC. *A new* and Organ Culture. 1991; 2:191-149. *Method for sugarcane breeding: Tissue Culture technique*. Taiwan. Sugar. 1971; 18(1):8-10.

4. Agricultural Year. *Industrial Crop Sugarcane. Agricultural Ataas of the Union of Myanmar*. Food and Agricultural Organization, Yangon, Myanmar, 2001-2002.
5. Nanda, Shing. *Rapid clonal multiplication ofsugarcane through tissue culture.* Plant Tissue Culture. 1994; 4:1-7.
6. Anita *et al*. *Efficient and cost effective micropropagation of two early maturing varieties of sugarcane (Saccharum spp).* Indian Sugar. 2000; 50:611-618.
7. Karim, *et al*. *In vitro* clonal propagation of sugar-cane (*Saccharum officicinarum*) variety Isol 31. Pak. J. Bid. Sci. 2002; 5:659-661.
8. Murashige T, Skoog F. *A revised medium for rapid growth andbioassays with tobacco tissue cultures.* Physiol Plant, 1962; 15:473-497.