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Research Article

IN VITRO PROPAGATION OF SELECTED SUGARCANE (*SACCHARUM OFFICINARUM* L.) VARIETIES (C-86-56) THROUGH SHOOT APICAL MERISTEM

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ABSTRACT
Sugarcane (*Saccharum officinarum* L.) is monocotyledonous crop plant that mostly propagates through conventional methods. However, conventional propagation lacks rapid multiplication procedures to commercialize newly released varieties within a short period of time. Hence, the objective of this work was to optimize in vitro micro propagation protocol for two sugarcane varieties (C-86-56) using apical meristem explants. The two varieties were cultured on MS medium supplemented with different concentrations of growth regulators on shoot initiation, multiplication and root induction stages. Analysis of variance (ANOVA) revealed that statistically significant difference in response to the various hormonal treatments with regard to the parameters measured. For initiation stage vars.B80-250 and B 78-550 performed best on 0.5 mg/l and 1.0 mg/l of BAP, respectively. On the other hand, multiplication stage was best in MS media enriched with 1. 5 mg/l BAP + 0.5 mg/l IBA and 2.0 mg/l BAP + 0.5 IBA as manifested in terms of mean number of shoots and mean shoot length for vars. B78-550 and B 80-250, respectively. With regard to root induction, best rooting response in terms of mean root number and mean root length was achieved best on 1/2 MS media enriched with 2.0 mg/l NAA + 0.5 mg/l BAP and 3.0 mg/l NAA + 0.5 BAP. Survival rate during acclimatization was best on coco peat media alone for both varieties of B78-550 and B 80-250 survived 93.33% and 96%, respectively. Lastly, factors causing, tissue dying, contamination and phenol exudation in the study should be further investigated.

Keywords: *Acclimatization, Apical Meristem, Explants, Growth Medium, In Vitro*

INTRODUCTION
Sugarcane (*Saccharum officinarum* L.) is a monocotyledonous crop plant that belongs to the family Poaceae (Magness, 2014). It is a clonally propagated crop from which multiple annual cuttings of stalks are typically obtained from each planting. This crop is especially vulnerable to diseases and propagation from cuttings facilitates the spread of pathogens and may results in epidemics (Schenck and Lehrer, 2000).

In certain countries, Sugarcane is eaten raw in minor quantities, while it is also used in the preparation of juices, animal feed and Mulch for gardens and farms. The main byproducts of the sugar industry are bagasse and molasses. Molasses, the chief by-product, is the main raw material for alcohol and for alcohol-based industries.

Excess bagasse is also used as raw material in the paper industry. In addition, bagasse is effectively used for co-generation of power in most sugar mills (Woodheap *et al*., 2006).

Sugarcane was introduced to Ethiopia during the Italian occupation of and commercial sugar production was started in 1954 from cane cultivated on a large scale by Dutch Company at Wonji (Mukerji and Associates, 2000). Sugar industry plays a great role in the Ethiopian socio-economy and provides employment opportunity for the people (Mukerji and Associates, 2000).

Propagation of sugarcane conventionally fails to produce adequate quantity because of susceptibility to disease. On the other hand, availability of adequate amount of quality and disease free planting materials within a short time is the major limiting factor to attain large scale sugarcane production using the conventional method of propagation and the yield of the existing few and old commercial sugar cane varieties is declining and some productive sugarcane varieties are also obsolete due to lack of alternative technologies for disease cleansing and rejuvenation (Tolera *et al*., 2014).

To solve the multitude problems of the conventional propagation method, the sugar industry utilizes the advantage of micro propagation technology, which is characterized by rapid multiplication to obtain disease free sugarcane varieties. The nutritional requirement for *in vitro* propagation protocol of sugarcane should be according to genotype and explants used (Soodi *et al.*, 2006); varieties (genotypes) of the same species respond differently to media (Roy, 2000). Besides rapid clonal propagation, sugarcane planting materials depend on the genotype and the plant growth regulators combinations used and needs to develop plant growth regulators combinations for each genotype. Similarly, plant growth regulators requirements for *in vitro* propagation responses vary from cultivar to cultivar in sugarcane (Raman *et al.*, 2005). The nutritional requirement for every sugarcane variety is specific and exact (Geetha and Padmanabhan, 2001). In addition, an efficient protocol is needed for any new variety or clone to get rapid shoot initiation, shoot multiplication, root induction & elongation (Godheja *et al.*, 2014). So, it is recommended that an efficient protocol is needed for every new variety or clone of sugarcane to get rapid callus induction, shoot initiation, shoot multiplication and root induction and elongation (Behera and Sahoo, 2009). Therefore, this study was carried out to develop or optimize *in vitro* protocol for mass propagation of two sugarcane varieties (B78-550 and B78-250) through apical meristem.

Objective of the Study

The general objective of this study was to develop optimization protocol for *in vitro* regeneration of two sugarcane varieties namely (B78-550 and B78-250) from shoot apical meristem explants.

Specific Objectives were

- To determine optimum concentrations of BAP hormone for initiation culture of apical meristem
- To determine best concentration of BAP and IBA hormones for shoot induction and multiplication.
- To determine best concentration of NAA and BAP hormones for root growth
- To evaluate survival rate of plantlets under greenhouse condition on different substrates

MATERIALS AND METHODS

Description of the Study Area

The study was conducted at Mekelle University; Plant Tissue Culture Laboratory which is located in Mekelle town, Tigray, Ethiopia that is located at latitude of 13°29′N, longitude of 39°28′E and altitude of 2076 meters above sea level (MARC, 2012).

Plant Material and Explant Preparation

Mother plants of the two varieties namely B78-550 and B80-250 that were used as a source of explants were raised from stem cuttings (setts) obtained from Welkayt Sugar Factory, Ethiopia. According to Shimelis *et al.*, (2014) those two Cuban varieties were imported to Ethiopia in 2006 and passed through agronomic performance evaluation. They were among the selected ones to be commercialized. Before planting, the setts were treated with hot water at 50^{o}C for 2 hours. Explant preparation were made following the method employed by Belay *et al.*, (2014), first the actively growing shoot tips with apical mersitem were collected from three months old mother plants to serve as explants. Shoot tips were cut from mother plants at the base with some nodes.

After trimming of the leaves, the shoot tips were taken to the laboratory for surface sterilization and explant preparation.

Trimmed shoot tips were washed thoroughly under running tap water, outer leaf sheath were removed and cut into about 10 cm length. Thereafter, the shoot tips further washed three times each for 15 minutes with tap water containing liquid soap solution and three drops of Tween-20. Then, explants were taken to laminar airflow chamber, immersed in 0.3 % (w/v) Kocide solution for 30 minutes followed by three times washing each for five minutes with sterile distilled water. The shoot tips were then rinsed in 70% alcohol for one minute and washed with sterile distilled water three times each for five minutes. Finally, the explants were treated with 10% (v/v) sodium hypochlorite solution (4% active chlorine) for 20 minutes. After discarding the sodium hypochlorite solution, the explants were washed with sterile

distilled water three times each for five minutes and the surface sterilized explants were excised and sized to 2.5 cm long for culturing (Belay *et al.,* 2014).

Culture Media Preparation

Full strength Murashige and Skoog (MS) (1962) basal medium were used as a culture medium. MS basal medium consisted of 30 g/l sucrose for initiation of apical meristem and shoot initiation and for rooting. The pH of the medium was adjusted to 5.8 using 1 N KOH and 1 N HCl before being gelled with 5.0 g/l agar and autoclaved at 121^{o}C, 15 psi for 20 minutes. While molten, the medium (40 ml) was dispensed into glass culture jar for culturing and stored under aseptic condition at + 4 ^{o}C until use for shoot initiation.

Initiation of Apical Meristem

For shoot initiation, the sterilized shoot tips were aseptically transferred to MS-medium prepared as indicated above with supplementation of PGR (BAP) at a concentration of 0.5, 1.0, 1.5 and 2.0 mg/l. MS medium without PGR were used as control. The treatments of initiation for both varieties were as follows

$T_{1=}$MS + 30 g/l sucrose + 5.0 g/l agar + 0.0 mg/l BAP
T_2=MS + 30 g/l sucrose + 5.0 g/l agar + 0.5 mg/l BAP
T_3=MS + 30 g/l sucrose + 5.0 g/l agar + 1.0 mg/l BAP
T_4=MS + 30 g/l sucrose + 5.0 g/l agar + 1.5 mg/l BAP
$T_{5=}$MS + 30 g/l sucrose + 5.0 g/l agar + 2.0 mg/l BAP

The explants were maintained in dark for 8 hours and light for 16 hours duration. The experiment was laid with three replicates.

Shoot Induction and Regeneration

For shoot initiation, MS basal medium supplemented with BAP in a concentration of 1.0, 1.5, 2.0 and 2.5 mg/l combined with 0.5 mg/l of NAA was used. MS basal medium without PGR, i.e., BAP and NAA were used as a control. Details of the treatments for both varieties were as follows:

$T_{1=}$MS + 30 g/l sucrose + 5.0 g/l agar + 0.0 mg/l BAP + 0.0 mg/l IBA
T_2=MS + 30 g/l sucrose + 5.0 g/l agar + 1.0 mg/l BAP + 0.5 mg/l IBA
T_3=MS + 30 g/l sucrose + 5.0 g/l agar + 1.5 mg/l BAP + 0.5 mg/l IBA
T_4=MS + 3 mg/l sucrose + 5.0 g/l agar + 2.0 mg/l BAP + 0.5 mg/l IBA
$T_{5=}$MS + 30 g/l sucrose + 5.0 g/l agar + 2.5 mg/l BAP + 0.5 mg/l IBA

Then after, cultures were maintained in a growth room at a temperature of 25 ± 2^{o}C under 16/8 hours light/dark photoperiod adjusted with fluorescent light having 2500 lux light intensity. The incubation chamber had relative humidity of 75-80%. Shoots were allowed to grow 2 to 4 cm and then transferred to rooting media. The experiment was laid out with a two factors treatment combinations each with three replicates.

Root Induction

Well grown 3-5 cm long shoots were aseptically transferred to 1/2 strength MS basal medium containing 0.0, 0.5, 1.0, 1.5, and 2.0 mg/l of NAA. The treatments of rooting for both varieties were as follows:

$T_{1=1/2}$ MS + 30 g/l sucrose + 5.0 g/l agar + 0.0 mg/l NAA + 0.0 BAP
$T_2=_{1/2}$ MS + 30 g/l sucrose + 5.0 g/l agar + 1.0 mg/l NAA+ 0.5 BAP
$T_3=_{1/2}$ MS + 30 g/l sucrose + 5.0 g/l agar + 2.0 mg/l NAA+ 0.5 BAP
$T_4=_{1/2}$ MS + 30 g/l sucrose + 5.0 g/l agar + 3.0 mg/l NAA+ 0.5 BAP
$T_{5=1/2}$ MS + 30 g/l sucrose + 5.0 g/l agar + 4.0 mg/l NAA+ 0.5 BAP

All the cultures were incubated at 25 ± 2^{o}C under 16/8 hours light/dark photoperiod adjusted with fluorescent light having 2500 lux light intensity. The relative humidity of the growth chamber was 75-80%. The experiment was laid out with NAA factor treatment each with three replicates.

Acclimatization

After four weeks of culture in a rooting media, well rooted *in vitro* plantlets were taken out gently from each PGR treatment bottle and washed under tap water to remove traces of agar that prevent the absorption of nutrients from the acclimatization culture substrates by roots. After this, plantlets were transferred to polystyrene tray that contains three different substrates, namely, coco peat, that is a

multipurpose growing medium made up of coconut husk, garden soil: sand: compost in the ratio of 1:1:1 and garden soil and cow dung in the ratio of 1:1.

Then, the polystyrene tray was arranged in completely randomized design in computerized green house with relative humidity (RH) gradually reduced from 90 to 60% and temperature of 30 to 31 °C for two weeks for primary acclimatization.

All the plantlets that survived the primary acclimatization were put in a direct sunlight and nursery shades were provided with adequate amount of water for secondary acclimatization and their performance were monitored for three weeks. Finally, plantlets that survived secondary acclimatization were transplanted to open field.

Data Collected

The following *in vitro* plant growth variables were recorded from this experiment and served as sources of quantitative data.

1. Percent of Initiated Culture: Percent of culture formed from the apical meristem explants after three weeks.

2. Mean number of Shoots: is the average number of dissectible shoots regenerated and induced from each cultured explants in each type of treatment.

3. Mean Number of Roots: is the average number of dissectible roots regenerated from each cultured shoot in each type of treatment.

4. Mean Length of Shoot: is the average length of shoots developed from the base of the shoot to the shoot apex before transferring to rooting media from each cultured explants. Lengths of the shoot were measured using sterilized ruler.

5. Mean Length of Root: is the average length of roots developed from each cultured explants. Length was measured on the 30^{th}day of transferring the shoot to the rooting media. Root length measurement was taken from the point that the root attached to the shoot to root tip.

6. Survival Rate: is the competence or the ability of the *in vitro* derived plantlets to endure in the *in vivo* condition for acclimatization. Data on this parameter was taken after one month after rooting transferred to greenhouse. Accordingly, the survival rate was calculated after three weeks as the ratio of plantlets survived to the total number of plantlets transferred to the greenhouse and expressed as percentage.

Data Analysis

Data were analyzed on the effect of treatments using SAS version 9.1 and means were compared using Fisher's Least Significant Difference (LSD) test at $p < 0.05$.

RESULTS AND DISCUSSION

Percent of Initiated Culture

Initiation culture from the apical meristem explants was observed within two weeks after inoculation of the explants on MS medium containing five different concentrations of BAP (0.0, 0.5,1.0 1.5 and 2.0 mg/l).

The results showed that shoot apical meristem culture initiation or establishment responses in the sugarcane varieties was dependent on the effect of sugarcane varieties (genotype) and BAP.

Among the different concentration BAP tested, sugarcane variety B78-550 gave the highest initiation culture responses (73.33%) on MS medium containing 1.0 mg/l BAP while B80-250 gave highest initiation culture responses (76.667 %) on MS medium supplemented with 0.5 mg/l BAP as shown in (Table 1).

This indicated that initiation response in these two varieties is different with respect to the concentration of BAP used.

In line with this, variation of initiation culture response to different concentration of hormones with variety of sugarcane was reported by Shimelis *et al*., (2014), Tilahun et *al*., (2014). Control showed no response for initiation in which all explants cultured on control (0.0 mg/l BAP) dried out after explantation.

Table 1: Effect of Different Concentrations of BAP on the Percent of Initiated Culture from Apical Meristem Explants of B78-550 and B80-250 Sugarcane Varieties

Treatments	Percentage	
	B78-550	**B80-250**
T_1(0.0 mg/l BAP)	———	———
T_2(0.5 mg/l BAP)	60.00[ab]	76.667[a]
T_3(1.0 mg/l BAP)	73.33[a]	66.667[ab]
T_4(1.5 mg/l BAP)	53.33[bc]	60.000 [c]
T_5(2.0 mg/l BAP)	43.33[c]	50.000[c]
Mean	4.600	5.0667
CV	19.4440	16.9016
LSD	1.6272	1.5579

Means followed within a column by the same letter are not significantly different at 5% significance level, CV=Coefficient of variance, LSD=Least significant different

Shoot Regeneration and Multiplication

Table 2: Different Shoot Parameters Measured for Apical Meristem Explants Treated with Different Concentrations of BAP Combined with 0.5 mg/l of IBA; Values are Mean ± SE, n=3

Sugarcane Varieties	Hormones (mg/l) BAP	IBA	No of Shoots per Expt.	Shoot Length(cm)
B80-250	0.0	0.0	———	———
	1.0	0.5	8.333[b]	3.4667[bc]
	1.5	0.5	8.667[ab]	5.0333[b]
	2.0	0.5	10.667[a]	8.5333[a]
	2.5	0.5	9.333[ab]	5.8333[b]
Mean			7.4000	4.5733
CV			16.3657	17.9602
LSD			2.2032	1.4943
B78-550	0.0	0.5	———	———
	1.0	0.5	7.333[b]	3.2667[b]
	1.5	0.5	9.333[a]	5.6333[a]
	2.0	0.5	8.667[ab]	4.4333[b]
	2.5	0.5	8.333[b]	3.1667[c]
Mean			6.7333	3.333
CV			15.3385	11.037
LSD			1.8789	0.663

Means followed by the same letter within each variety are not significantly different at 5% significance level, No=Number, Expt. =Explant, Wt. =Weight, CV=Coefficient of variance, LSD=Least significant different.

Mean Number of Shoots

Number of shoot/explant was significantly higher in PGRs treated explants than PGRs free cultured explants. Number of shoot /explants was also significantly varied between PGRs treatments with highest number (10.667) counted at 2.0 mg/l of BAP combined with 0.5 mg/l of IBA for variety B80-250 and 9.333 shoot/explants for variety B78-550 at 1.5 mg/l of BAP combined with 0.5 mg/l of IBA (Table 2). Previously, (Tarique *et al.*, 2010*)* reported that 1.0 mg/l BAP + 0.5 mg/l NAA and 1.0 mg/l BAP + 0.5 mg/l IBA showed the best result for induction and multiplication of shoots for sugar cane varieties of B52-298 and NCO-334, respectively. This shows that different varieties of sugar cane respond differently to different types and concentrations of PGRs, suggesting unique optimization for better performance.

Moreover, Genotype specific response to number of shoot regeneration was reported by Behara and Sahoo (2009).

Shoot number /explant appeared to increase with increasing concentration of BAP up to 2.0 mg/l. It has been reported that a high level of cytokinin in combination with a low auxin level was essential for the differentiation of adventitious shoots in sugarcane (Belay *et al*., 2014). However, it was observed that mean number of shoots per explants was found to decline with further increase in the concentration of BAP beyond optimum (2.0 mg/l BAP) for both varieties. This finding agrees with Khalafalla *et al*., (2007) who reported that BAP at the concentration of 5.0 mg/l gives low number of shoot per explants and concluded that shoot number decreases as BAP concentration increases beyond optimum. Increasing trend in shoot number per explants up to optimum level is due to the fact that cytokinin (BAP) stimulates protein synthesis and participates in cell cycle control in a cell division (George *et al*., 2008).

Effect of Growth Regulators on Shoot Length

Average shoot length was significantly higher in PGRs treated explants than explants culture on PGRs free media. Shoot length also showed significant difference between PGRs treatments. Shoots cultured on MS media containing 2.0 mg/l BAP and 0.5 mg/l IBA showed significantly higher mean shoot length (8.533) compared to all other treatments for var. B80-250. For var. B78-550 highest mean shoot length (5.633) measured was on MS medium containing 1.5 mg/l BAP and 0.5 mg/l IBA (Table 2). Similar to this result, Shimelis *et al*., (2014) reported maximum shoot length 8.4 ± 0.008 for Cuban sugar cane variety C 86-12 when cultured on BAP (1.5 mg/l) + kin (0.5 mg/l). Behera and Sahoo, (2009) also reported that maximum shoot length of 6.2 ± 0.37 and 4.0 ± 0.61 under BAP (2.0 mg/l) + IBA (0.5 mg/l) and BAP (2.0 mg/l) + IBA (1.0 mg/l) for two sugarcane varieties namely B52-298 and NCO-334, respectively. On the other hand, it was observed that shoot length was found to decline with the increase in the concentration of BAP beyond optimum (2.0 mg/l BAP). This findings agrees with that of Bhatia *et al*., (2005) who explained that increasing the concentration of the PGRs over optimum supplements may lead to negative effects on the morphology of the *in vitro* shoots.

Effect of Growth Regulator (NAA) on Root Induction

Number of Roots per Shoots

Number of root/shoot was significantly higher in PGR treated shoot than PGR free cultured shoots. Number of roots was also significantly varied between PGR treatments with highest number (12.667) counted at 3.0 mg/l of NAA+ 0.5 mg/l BAP for variety B80-250 and 9.0 for variety B78-550 at ½ MS medium supplemented with 2.0 NAA+ 0.5 mg/l BAP (Table 3). This result can be complemented by a number of previous studies. For example, Behera *et al.*, (2006) found highest number of roots per micro shoots (13.4 ± 1.5) on ½ MS medium supplemented 2.5 mg/l NAA for sugar cane varieties B52-298. The above result contradicts to Shimelis *et al*., (2015) reported that maximum root/shoot (17.8) on ½ MS medium supplemented with 5.0 mg/l NAA for C 86-12 sugar cane variety.

Effect of Growth Hormones on Root Length

Root length was significantly ($p < 0.05$) affected by different concentrations of NAA supplemented to ½ MS medium for both varieties (Table 3). Variety B80-250 produced maximum root length (5.0667 cm) on half strength MS media containing 3.0 NAA + 0.5 mg/l BAP. But, variety C 86-12 produced maximum root length (4.7667 cm) on half strength MS media containing 2.0 mg/l NAA+ 0.5 mg/l BAP. In line with this, Belay (2016) reported root length of 3.2 ± 0.25 cm when grown on ½ strength MS medium supplemented with 1.0 mg/l NAA alone for N14 sugarcane variety. Mangrio *et al*., (2009) obtained average root length of 2.5 cm on ½ MS media supplemented with 3.0 mg/l NAA for sugarcane Variety NCO-334. The effect of variations in the concentrations and combination of the same hormone in most of the cited literatures and in the present work is almost entirely due to variation in the varieties of sugarcane tested. That is why it is of paramount importance to optimize genotype specific *in vitro* propagation protocols for every variety.

Survival Rate in Green House during Acclimatization

In vitro induced shoots are very delicate and cannot resist sudden environmental changes that may damage the plantlets unless they are gradually adapted to the new environment. Thus, acclimatization is

essential to enable the rooted plantlets to adapt the natural environment in *ex vitro* conditions at controlled temperature and humidity of greenhouse conditions. In the acclimatization stage of this experiment, a total of 90 and 75 well rooted plantlets for variety B80-250 and B78-550, respectively were transferred to greenhouse containing substrates namely, coco peat alone, that is a multipurpose growing medium made up of coconut husk; garden soil: sand: compost in the ration of 1:1:1 and garden soil and cow dung in the ration of 1:1. Then, the polystyrene tray was arranged in greenhouse with relative humidity (RH) gradually reducing from 90 to 60% and temperature of 30 to 31 °C for two weeks for primary acclimatization. Generally, the acclimatization phase of this experiment revealed that there was a difference in survival rate due to substrate nature and varietal difference. Both varieties had the highest survival value when grown on coco peat alone. On this media substrate, survival rate was 93.33% and 96% plantlet for B78-550 and B80-250 sugarcane varieties, respectively as shown (Table 4).

Table 3: Effect of Different Concentrations of NAA on Rooting Responses of B78-550 and B78-250 Sugarcane Varieties; Values are Means and n=3

Sugarcane Varieties	Hormone(mg/l)		No. of Roots per Shoot	Root Length(cm)
	NAA	BAP		
B80-250	0.0	0.0	———	———
	1.0	0.5	7.667^{b}	3.7667^{b}
	2.0	0.5	9.000^{b}	3.833^{b}
	3.0	0.5	12.667^{a}	5.0667^{a}
	4.0	0.5	12.000^{a}	3.700^{b}
Mean			8.2667	3.1823
CV			17.668	22.5452
LSD			2.6572	1.3043
B78-550	0.0	0.0	———	———
	1.0	0.5	9.000^{a}	4.7667^{a}
	2.0	0.5	5.000^{c}	4.500^{ab}
	3.0	0.5	7.000^{b}	3.333^{b}
	4.0	0.5	5.667^{bc}	3.633^{ab}
Mean			5.333	3.2466
CV			15.3093	21.0710
LSD			1.4854	1.2446

Means followed by the same letter within each variety are not significantly different at 5% significance level. No=number, Wt. =weight, CV=Coefficient of variance, LSD=Least significant different.

Table 4: Effect of Different Medium Substrates on the Survival of *in Vitro* Regenerated Plantlets of the Two Varieties during Acclimatization Stage in Green House

Types of Sugarcane Varieties	Types of Culture Medium	Total No of Plantlet Transferred	Survived Plantlets	Percent of Survived Plantlets
B80-250	Coco peat only	30	28	93.33%
	Garden soil: sand: compost (1:1:1)	30	18	60%
	Garden soil: cow dung (1:1)	30	20	66.7%
B78-550	Coco peat only	25	24	96%
	Garden soil: sand: compost (1:1:1)	25	15	60%
	Garden soil: cow dung(1:1)	25	16	64%

No=Number

Figure 1: Preparation of Explants from Matured Shoot Apical Meristem

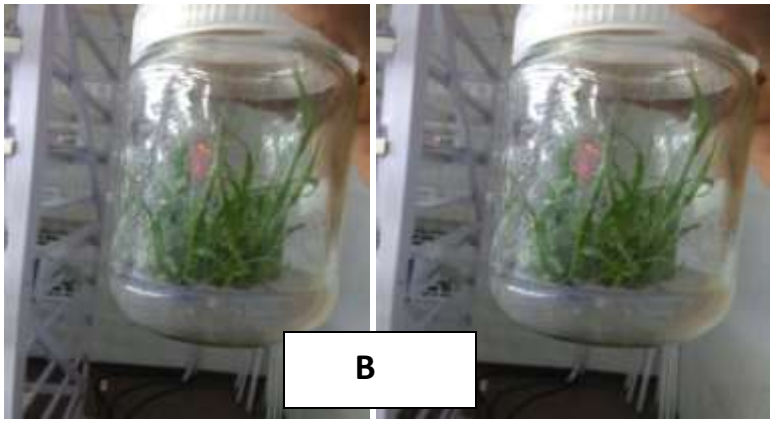

Figure 2: Examples of Best Cultures on Shooting Stage

Figure 3: Best Plantlets in Green House on Coco Peat Medium (Survived and Died Plantlets)

Summary, Conclusion and Recommendation

Summary and Conclusion

Sugarcane, as a globally important industrial crop, is a main of sugar, ethanol and other important by products. Hence, due consideration to the use of advanced technologies for sugarcane production is mandatory to obtain the unfolded benefits tapped from the crop. Thus, multiplication of sufficient quality of seeding material is needed more than ever before. However, in sugarcane seeding material

multiplication usually takes up to 10 years following conventional method; besides the method allows continuation of diseases over vegetative cycles, which leads to drastic yield and quality reduction. To overcome the problem, *in vitro* propagation that enables rapid and large scale production of disease free planting material as being exercised with different crops is a prerequisite.

Based on this fact plant regeneration protocol was optimized in this study through direct organogenesis for two commercially important Cuban originated sugarcane varieties (B78-550 and B80-250) using apical meristem explants. Accordingly, the information below was obtained. For initiation stage of apical meristem explants and more initiated culture var. B80-250 best perform on 0.5 mg/l BAP mg/l, while var. B78-550 best perform on 1.0 mg/l of BAP.

Shoot parameters were also highly influenced by varieties and the type and combinations of various growth regulators. The effect of varieties and hormones combinations was highly significant ($p \leq 0.05$) on average values of shoot parameters. The highest number of initiated explants cultures regenerated more number of shoots and optimum shoot length were observed in *var*. B80-250 on full MS media supplemented with 2.0 mg/l BAP + 0.5 mg/l IBA, whereas maximum percentage of initiated explants cultures regenerated more number of shoots and optimum shoot length were recorded on 1.5 mg/l BAP + 0.5 mg/l IBA for B78-550 variety. Roots regenerated 10 to 15 days after micro shoots were transferred to root induction media for both varieties. Highly significant ($p \leq 0.05$) differences were observed among treatments and varieties were also highly significant ($p \leq 0.05$). The highest percentage of shoots regenerated number of roots and root length were recorded for *var*. B78-550 on half strength MS media containing 2.0 mg/l NAA+ 0.5 mg/BAP, whereas for *var*. B80-250 maximum percentage of number of root and root length was recorded on half strength MS media containing 3.0 mg/l NAA + 0.5 mg/l BAP. As to the acclimatization response, relatively highest survivability percentages were recorded on coco peat media substrate as compared to the other medium substrates used. Besides from the two varieties B80-250 survived higher percentage (96%) than B78-550 (93.33%) on coco peat media.

The results clearly indicated the importance of evaluating individual variety to optimize a given tissue culture protocol. In other words, genotypic specificity was highly reflected in all of the parameters tested. Genotypic specificity has been reviewed in the literature review part of this paper and many researchers have imposed the evaluation of individual variety to recommend a tissue culture protocol.

Recommendation

Based on the above findings, the following recommendations are made for further investigation of *in vitro* culture of the two varieties.

- Further studies are needed using other hormones such as Kn, 2,4-D, IAA, IBA with different concentration and interaction effects for observing their ability to induce shoots and roots for the reproducibility of the protocol optimization through direct or in direct organogenesis.
- Factors causing contamination, low acclimatization, tissue dying, and phenol exudation in the study should be further investigated.
- It is necessary to study the performance and genetic stability of the *in vitro* regenerated seedlings after transplanting in the field necessary.
- To enhance the acclimatization rate of *in vitro* developed plantlets in the glasshouse, various methods have to be manipulated.
- To sum up, the present study has developed protocol optimization for *in vitro* micro propagation of new Cuban origin *Saccharum officinarum* L. varieties (B78-550 and B80-250) using apical meristem explants through direct organogenesis. Hence, it is beneficial to use this developed *in vitro* micro propagation protocol as a best follow up to large scale propagation to generate large number of seedlings in short period of time.

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