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Protocol optimization for *in vitro* mass propagation of two sugarcane (*Saccharum officinarum* L.) clones grown in Ethiopia

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The present study was initiated to optimize *in vitro* protocol for mass propagation of two commercial sugarcane clones (Co 449 and Co 678) grown in Ethiopia through shoot tip culture. Experiments on shoot multiplication and rooting were laid out in a completely randomized design with factorial treatment arrangements. Shoot tips were surface sterilized with 5% active chlorinated Berekina for 25 min and initiated on Murashige and Skoog (MS) medium supplemented with 2 mg L^{-1} 6- benzylamino purine + 0.5 mg L^{-1} indole-3- butyric acid. For *in vitro* multiplication, aseptically initiated shoot tips were treated with different concentrations and combinations of BAP and Kin using either sucrose or table sugar in separate experiments. For root induction, regenerated shoots were transferred onto half MS medium supplied with 6% sucrose or table sugar and different concentrations and combinations of IBA and NAA. With regard to shoot multiplication, genotype Co449 showed maximum regeneration frequency of 80% with 7.87 ± 1.06 shoots per explants on MS medium with 3% sucrose and 2 mg L^{-1} BAP + 0.25 mg L^{-1} Kin. On the same carbon source, genotype Co678 showed the highest multiplication frequency of 90% with 9.10 ± 0.10 shoots per explant on medium supplied with 2 mg L^{-1} BAP + 0.5 mg L^{-1} Kin. On MS with 4% table sugar and 2 mg L^{-1} BAP + 0.25 mg L^{-1} Kin, 80% of the transferred explants of genotype Co449 produced multiple shoots with an average number of 7.61 ± 0.10 shoots per explant while genotype Co678 showed the highest regeneration frequency (86.67%) with mean shoots number per explant of 8.36 ± 0.04 on medium supplemented with 2 mg L^{-1} BAP + 0.5 mg L^{-1} Kin. Half MS + 6% sucrose + 2.5 mg L^{-1} IBA induced the highest rooting (86.67%) with an average root number per shoot of 16.53 ± 0.02 in Co449 cultures. In genotype Co678, ½ MS + 6% sucrose + 5 mg L^{-1} NAA induced the highest rooting response of 80% with an average root number per shoot of 13.17 ± 0.29. Equivalent rooting responses were recorded on half MS medium supplemented with 6% table sugar. On½ MS with 6 % table sugar, 2.5 mg L^{-1} IBA induced the highest rooting response (86.67%) and root number (15.93 ± 0.81) in genotype Co449 while 5 mg L^{-1} NAA gave the maximum (80%) rooting with average roots per shoot of 13.93 ± 0.81 in genotype Co678. Rooted shoots were transplanted in the green house for hardening and different survival rate was recorded.

Key words: *Saccharum officinarum*, multiplication, rooting, sucrose, table sugar, *in vitro*.

INTRODUCTION

Sugarcane (*Saccharum officinarum L.*) is a herbaceous perennial crop plant that belongs to the family Poaceae (Singh, 2003; Sharma, 2005; Cha-um et al., 2006). It has chromosome number of 2n = 80 (Daniels and Roach, 1987; Asano et al., 2004). It had been thought to be evolved in Asia, probably the island of New Guinea (Singh, 2003). Today, sugarcane is cultivated in over 110 countries and 50% of the production occurs in Brazil and India (FAO, 2008). Sugarcane contributes nearly 70% of global annual sugar production (Sengar, 2010).

Sugarcane is one of the most widely grown crops in Ethiopia, even though the history is not well-documented when it was introduced (Assefa, 2006). According to Tafesse and Haile-Michael (2001), the Dutch Company, Handles-Vereenging Amsterdam (HVA) pioneered commercial cultivation of sugarcane in Wanji, Ethiopia in 1954/55. In Ethiopia, sugarcane cultivation has multipurpose. The sugar juice is used for making sugar. Molasses (thick syrupy residue) is used in the production of ethanol (blended for motor fuel) and as livestock feed. The bagasse (fibrous portion) is burned to provide heat and electricity for sugar mills and green tops can be used as cattle feed. Furthermore, sugar factories being located in rural areas, they generate employment opportunity for thousands of people at various stages of production (Girma and Awulachew, 2007). At the moment, there are three large scale sugar establishments in Ethiopia: Wonji/Shoa, Matahara and Fincha, producing a total of about 300,000 tons of sugar and eight million liter of ethanol per year (Feyissa et al., 2010). However, the current production is not satisfying the existing domestic sugar and its by-products demand. As a result, establishment of 10 new sugar factories and expansion of the existing ones are underway with the aim of producing 2.5 million tons sugar and 304 million liter ethanol at the end of the growth and transformation plan (GTP) (Ambachew and Firehun, 2010). Currently, sugarcane is cultivated on about 33,777 ha of land in the country, of which 30,157 ha is government owned and the remaining portion is private owned. It is planned to increase the government owned sugarcane plantation to 200,000 ha at the end of the Ethiopian growth and transformation plan (Feyissa et al., 2010). The increase in plantation area creates high demand of good planting material to be available and these calls for a means that can provide planting material in large scale and within short period of time.

However, the current conventional seed cane production method where stem cuttings with two or three nodes used as planting material, has various limitations. The seed multiplication rate is too low (1:6 to1:8) which makes the spread of newly released varieties slow, taking over 10 years to scale up a newly released variety to the commercial level (Cheema and Hussain, 2004; Sengar, 2010), and also facilitates the spread of pathogens and may result in epidemics (Schenck and Lehrer, 2000). Moreover, the method requires large nursery space (Sundara, 2000). Therefore, developing an efficient propagation system for mass multiplication of sterile sugarcane planting material of selected variety is of paramount importance. In line with this, application of micropropagation techniques for the propagation of sugarcane has the benefits of rapid propagation of new cane varieties, reduction in seed use, regeneration of large number of true to type plantlets from a small tissue, elimination of pathogens and storage of plant germ-plasm under aseptic condition (Ali et al., 2004; Gosal et al., 2006; Khan et al., 2006). However, implementation of micropropagation technology is influenced by many factors such as production cost and knowhow of micropropagation protocols, which make the technology expensive and unaffordable by less developed countries (Demo et al., 2008).

In plant culture, no two genotypes give similar response under a given set of culture conditions (Nehara et al., 1989; 1990a). It often requires testing of various type, concentration and mixture of the growth regulators during the development of a tissue culture protocol for a new plant tissue (Bhojwani and Razdan, 1996). With this reason, standardization of protocols for *in vitro* multiplication of sugarcane through callus culture, axillary bud and shoot tip culture have been reported by many authors (Barba et al., 1978; Bakesha et al., 2002; Alam et al., 2003; Ali et al., 2008; Behara and Sahoo, 2009; Khan et al., 2009).

So far, there is no report that is adopted for *in vitro* mass propagation of sugarcane genotypes grown in Ethiopia, and due to this the country is not getting advantage of this modern technology. Therefore, the present study was conducted to develop/optimize *in vitro* protocol for mass propagation of two sugarcane clones (Co 449 and Co 678) grown in Ethiopia through shoot tip culture.

MATERIALS AND METHODS

The study was conducted at plant tissue culture laboratory of Jimma University College of Agriculture and Veterinary Medicine, Ethiopia. Two sugarcane genotypes, Co449 and Co678, were considered in this study. They were obtained from Matahara Sugar Estate under the license of Ethiopian Sugar Corporation. To reduce explants sourced contamination, the stock plants were raised by planting seed canes under greenhouse condition. For *in vitro* studies, shoot tips were excised from tops of three to four months-old actively growing sugarcane raised in the greenhouse. The

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Table 1. ANOVA summary of effect of BAP and Kin on shoot multiplication and shoot growth on MS with sucrose.

Source of variation	DF	Mean square			
		Multiplication rate	Number of shoots/explant	Shoot length	Number of leaves per hoot
Gen	1	56.02***	2.74***	271.85***	3.14***
Kin	2	3672.28***	56.81***	1491.82***	12.81**
BAP	4	13840.35***	90.19***	2079.67***	36.17***
Gen *Kin	2	199.53***	1.23***	38.50***	0.86***
Gen *BAP	4	190.72***	0.35*	18.91***	0.49***
Kin *BAP	8	51.54***	1.20***	158.63***	0.56***
Gen *Kin *BAP	8	149.19***	1.48***	6.60***	0.25***
CV (%)		5.48	8.66	3.04	7.23

***= Very highly significant (P <0.0001) at α=0.05 significance level,*= significant (P = 0.03) at α=0.05 significance level, DF= Degree of freedom, Gen = Sugarcane genotypes, Kin=Kinetin and BAP =6-Benzylaminopurine

leaves were removed and the shoot blocks were taken to the laboratory. In the laboratory, surrounding leaf sheaths were carefully removed one by one until the inner white sheaths were exposed. Then, 10 cm long tops were collected by cutting off at the two ends, locating the growing point somewhere in the middle of the top. The shoot tip blocks were washed under running tap water for 30 min with soap solution which was followed by treating with 0.3% Kocide (fungicide solution) for one and half hour under laminar air flow. After decanting the kocide, shoot tip blocks were rinsed three times with sterile distilled water and then treated with 70% ethanol for 30 s. Then, after three times rinsing with sterile distilled water, the explants were treated with Berekina (with 5% active ingredient of chlorine) for 25 min. To increase efficacy, two drops of Tween-20 solution was added into Berekina solution. Decanting the sterilizing solution under safe condition, the explants were washed three times each for 5 min with sterile distilled water and were left for 10 min to make their surface dry. Then, leaf sheaths damaged during sterilization were removed by using sterilized forceps. Finally, 2 cm long shoot tips were excised with sterilized scarples and cultured on MS basal medium supplemented with 2 mg L^{-1} BAP + 0.5 mg L^{-1} IBA, 3% sucrose, 0.8% agar (Bakasha et al., 2002). For shoot multiplication, aseptically initiated 3 cm long cultures were transferred to MS basal medium supplemented with 3% sucrose or 4% table sugar, 0.8% agar and varying concentrations of BAP (0.0, 0.5, 1.0,1.5 and 2.0 mg L^{-1}) and Kin (0.0, 0.25 and 0.5 mg L^{-1}) in factorial combination. To avoid the carry over effect of multiplication media on *in vitro* rooting, multiplied shoots were maintained on plant growth regulators free MS basal medium for the next two weeks. The rooting response of *in vitro* regenerated shoots was considered on half strength MS basal medium supplemented with 6% sucrose or 6% table sugar, 0.8% agar and different concentration of IBA (0.0, 1.25, 2.5, 3.75 and 5 mg L^{-1}) in factorial combination with NAA (0.0, 1.25, 2.5, 3.75 and 5 mg L^{-1}). In all the cases, 15 explants were cultured per treatment combination and cultures were maintained in growth chamber conditions of temperature of 25 ± 2°C, 16 h light photoperiod, relative humidity of 70-80%, and fluorescent light intensity of 2500 lux. Plantlets with well developed shoots and roots were transplanted in plastic pot containing a mixture of sieved river sand, forest soil and well decomposed farm yard manure (FYM) in a 1:1:1 ratio and transferred to greenhouse for hardening. The transplanted plantlets were kept in greenhouse under shade of polyethylene sheets for 14 days and were sprayed with water two to three times every day in the first month and once in the remaining time. After 45 days, observation on percentage of plantlets that were successfully acclimatized was recorded. For the multiplication experiment, percent of regenerated explants, average number of shoots per explant, average shoots length (cm) and average number of leaves per shoots were recorded for each treatment combination after 30 days of transfer. Regarding root regeneration experiment, percentage of microshoots rooted, average number of roots per microshoot, and average root length (cm) were recorded after 30 days of culture transfer from plant growth regulators (PGR) free MS medium on to root induction medium. All collected data were subjected to three way ANOVA using SAS software version 9.2 (SAS Institute Inc., 2008). In all the cases, statistical significance was computed at α = 5% and treatment mean separation was done using procedure of REGWQ (Ryan, Elinot, Gabriel, and Welsh) multiple range test.

RESULTS AND DISCUSSION

Effect of BAP and Kin on shoot multiplication of sugarcane *in vitro* cultures on MS medium with sucrose

ANOVA showed that genotype, kinetin, BAP and their interactions had very high significant (p<0.0001) effects on shoot multiplication frequency, average shoots number, average shoot length and average number of leaves per shoot (Table 1). Interaction of genotype (Gen), Kinetin (Kin) and BAP (Gen*Kin*BAP) revealed that all the three factors are dependent on each other for *in vitro* multiplication of sugarcane.

For both genotypes, the lowest multiplication response (0%) was recorded on MS basal medium devoid of BAP and Kin while multiple shoot formation occurred in the presence of BAP and Kin (Table 2). The use of 2 mg L^{-1} BAP without Kin produced 4.56 shoots per explants in genotype Co449, which was significantly improved to 7.87 shoots by addition of 0.25 mg L^{-1} Kin (Table 2). Similar trend of increase in number of shoots per explants was observed for genotype Co678 with an inclusion of Kin. These showed the importance of including Kinetin along with BAP in shoot multiplication media. This essentially indicates that the use of cytokinins (BAP and Kin) have a positive effects and play important role in multiplication of sugarcane cultures. In fact, cytokinins (BAP and Kin) stimulate protein synthesis and participate in cell cycle control and if added into shoot culture media,

Table 2. Effect of BAP and Kin on *in vitro* shoot multiplication of sugarcane cultures on MS with sucrose.

Genotype	Hormone combination		Percentage of Explants multiplied	Number of Shoots/Explant (Mean ± SD)	Shoot length (cm) (Mean ± SD)	Number of Leaves/Shoot (Mean ± SD)
	Kin (mg/L)	BAP (mg/L)				
	0	0	0.00^{o}	$0.00^{n} \pm 0.00$	$0.00^{k} \pm 0.15$	$0.00^{s} \pm 0.00$
	0	0.5	6.67^{n}	$1.78^{klm} \pm 0.77$	$3.40^{ij} \pm 0.15$	$2.22^{opq} \pm 0.19$
	0	1	20.00^{l}	$2.67^{ijk} \pm 0.58$	$4.10^{ij} \pm 0.10$	$3.00^{lmn} \pm 0.00$
	0	1.5	46.67^{h}	$3.51^{ih} \pm 0.16$	$5.10^{hg} \pm 0.06$	$3.32^{j-m} \pm 0.34$
	0	2	73.33^{d}	$4.56^{efg} \pm 0.38$	$6.20^{bc} \pm 0.20$	$4.67^{b-e} \pm 0.00$
	0.25	0	6.67^{n}	$0.33^{n} \pm 0.58$	$3.13^{j} \pm 0.06$	$1.57^{qr} \pm 0.12$
	0.25	0.5	26.67^{k}	$2.33^{ijk} \pm 0.00$	$4.27^{j} \pm 0.12$	$2.87^{mno} \pm 0.12$
Co449	0.25	1	40.00^{i}	$2.88^{ij} \pm 0.51$	$5.23^{efg} \pm 0.06$	$3.44^{i-m} \pm 0.51$
	0.25	1.5	66.66^{e}	$5.16^{def} \pm 0.12$	$6.40^{def} \pm 0.10$	$4.10^{d-i} \pm 0.17$
	0.25	2	80.00^{c}	$7.87^{b} \pm 1.06$	$6.33^{bc} \pm 0.21$	$5.44^{a} \pm 0.19$
	0.5	0	13.33^{m}	$1.66^{lm} \pm 0.58$	$4.30^{ij} \pm 0.10$	$1.50^{r} \pm 0.00$
	0.5	0.5	20.00^{l}	$4.11^{gh} \pm 0.19$	$5.43^{def} \pm 0.15$	$2.83^{mno} \pm 0.29$
	0.5	1	46.67^{h}	$5.44^{dce} \pm 0.19$	$5.90^{def} \pm 0.10$	$3.89^{f-j} \pm 0.29$
	0.5	1.5	73.00^{d}	$6.23^{c} \pm 0.08$	$6.53^{cde} \pm 0.06$	$4.75^{bcd} \pm 0.21$
	0.5	2	80.00^{c}	$7.33^{b} \pm 0.03$	$6.37^{ab} \pm 0.25$	$5.03^{abc} \pm 0.15$
	0	0	0.00^{o}	$0.00^{n} \pm 0.00$	$0.00^{k} \pm 0.00$	$0.00^{s} \pm 0.00$
	0	0.5	13.33^{m}	$1.56^{lm} \pm 0.19$	$3.47^{ij} \pm 0.06$	$2.27^{op} \pm 0.06$
	0	1	26.67^{k}	$2.11^{j-m} \pm 0.38$	$4.27^{hg} \pm 0.15$	$3.20^{k-n} \pm 0.17$
	0	1.5	40.00^{i}	$2.67^{ijk} \pm 0.58$	$5.30^{def} \pm 0.26$	$4.00^{e-j} \pm 0.00$
	0	2	60.00^{f}	$5.63^{dc} \pm 0.04$	$6.23^{abc} \pm 0.25$	$4.33^{c-f} \pm 0.58$
	0.25	0	13.33^{m}	$1.33^{m} \pm 0.00$	$3.07^{ij} \pm 0.12$	$2.03^{pqr} \pm 0.58$
	0.25	0.5	20.00^{l}	$3.56^{ih} \pm 0.69$	$3.77^{hi} \pm 0.06$	$3.54^{h-m} \pm 0.21$
Co678	0.25	1	33.33^{j}	$4.44^{gf} \pm 0.19$	$5.33^{def} \pm 0.15$	$4.22^{d-h} \pm 0.37$
	0.25	1.5	60.00^{f}	$5.39^{dce} \pm 0.10$	$6.50^{ab} \pm 0.61$	$4.31^{d-g} \pm 0.30$
	0.25	2	86.00^{b}	$7.21^{b} \pm 0.02$	$6.93^{a} \pm 0.12$	$4.53^{b-f} \pm 0.50$
	0.5	0	20.00^{l}	$2.22^{j-m} \pm 0.19$	$3.13^{ij} \pm 0.06$	$2.54^{nop} \pm 0.21$
	0.5	0.5	46.67^{h}	$3.36^{ih} \pm 0.69$	$4.27^{hg} \pm 0.12$	$3.67^{g-l} \pm 0.00$
	0.5	1	53.33^{g}	$5.42^{dce} \pm 0.21$	$5.67^{cd} \pm 0.21$	$4.70^{b-e} \pm 0.26$
	0.5	1.5	60.00^{f}	$7.11^{b} \pm 0.13$	$6.63^{ab} \pm 0.06$	$5.17^{ab} \pm 0.29$
	0.5	2	90.00^{a}	$9.10^{a} \pm 0.10$	$6.83^{a} \pm 0.12$	$5.67^{a} \pm 0.00$
CV (%)			**5.48**	**8.66**	**3.04**	**7.24**

Means in column with the same letter are not significantly different by Ryan-Einot-Gabriel-Welsch Multiple Range Test at α= 5% significant level.

stimulate lateral bud growth and thus causing multiple shoot formation by breaking shoot apical dominance (Trigiano and Gray, 2005; George and Klerk, 2008).

Among the various concentrations and combinations, MS medium supplemented with 2 mg L^{-1} BAP + 0.25 mg L^{-1} Kin and 2 mg L^{-1} BAP + 0.5 mg L^{-1} Kin showed maximum shoot multiplication frequency (80%) in sugarcane genotype Co449 (Table 2). On MS medium + 2 mg L^{-1} BAP + 0.25 mg L^{-1} Kin, genotype Co449 gave an average of 7.87 ± 1.06 shoot per explant with shoot length of 6.33 ± 0.21 cm and leaf number of 5.44 ± 0.19 per shoot; raising concentration of Kin to 0.5 mg L^{-1} on the same media composition for Co449 did not result in better number of shoots per explants.

On the other hand, genotype Co678 showed the highest shoot multiplication on MS medium supplemented with 2 mg L^{-1}BAP + 0.5 mg L^{-1} Kin where 90% of the transferred cultures showed multiple shooting with an average of 9.10 ± 0.10 shoots per culture, 6.83 ± 0.12 cm shoot length and 5.67 ± 0.00 leaves per shoot. MS + 2 mg L^{-1} BAP + 0.25 mg L^{-1} Kin, which showed the highest number of shoots per explants in genotype Co449, resulted in 7.21 shoots per explants with 86% of the explants showing multiple shoot formation for Co678 and thus this medium can be considered as the second best medium combination for this genotype.

The current result is consistent with other *in vitro* multiplication reports of sugarcane using 3% sucrose in MS

Table 3. ANOVA summary of effect of BAP and Kin on shoot multiplication and shoot growth on MS with table sugar.

Source of variation	DF	Mean square			
		Multiplication rate	Number of shoots/explant	Shoot length	Number of leaves / shoot
Gen	1	18.67***	3.67** *	6.51***	3.90***
Kin	2	2794.71***	49.18***	35.17***	11.97***
BAP	4	11836.35***	78.08***	48.25***	36.67***
Gen * Kin	2	454.69***	0.30**	0.17***	0.67***
Gen *BAP	4	118.11***	0.96***	0.44***	0.57***
Kin * BAP	8	87.48***	1.35***	3.58***	0.69***
Gen * Kin *BAP	8	72.49***	1.46***	0.16***	0.28***
CV (%)		5.28	5.77	3.03	6.81

***= Very highly significant (P <0.0001) at α=0.05 significance level,** = highly significant (P = 0.0044) at α=0.05 significance level, DF= Degree of freedom, Gen = Sugarcane genotypes, Kin=Kinetin and BAP =6-Benzylaminopurine.

medium. Khan et al. (2009) found maximum (7) shoot number per explants with 8.5 cm shoot length on MS medium supplemented with 1.0 mg L^{-1} BAP and 0.5 mg L^{-1} Kin in sugarcane variety CP-77-400. In addition, they observed 6 shoots per explants on MS medium with 1.0 mg L^{-1} BAP + 0.1 mg L^{-1} Kin in sugarcane variety CPF-237 and 8 shoots per explants on MS media supplemented with 1.0 mg L^{-1} BAP + 0.5 Kin in sugarcane variety HSF-240. In the present study, an average shoot number of 5.44 in genotype Co449 and 5.42 shoots in genotype Co678 were observed on MS medium with 1.0 mg L^{-1} BAP + 0.5 mg L^{-1} Kin which are almost in line with the report of Khan et al. (2009) at this level. Singh (2003) observed an average of 12.33 shoots per explants on MS medium supplemented with 1.5 mg L^{-1} BAP and 0.5 mg L^{-1} Kin. Bakesha et al. (2002) reported multiple shoots from shoot tip explants of sugarcane cultured on MS medium supplemented with BAP (0.5 - 2.0 mg L^{-1}) and Kin (0.5 mg L^{-1}). In terms of shooting frequency, the present result agree with the report of Behera and Sahoo (2009) and Biradar et al. (2009) who respectively reported 92% and 79.64% shoot multiplication frequency in sugarcane *in vitro* micropropagation. Therefore, 2 mg L^{-1} BAP + 0.25 mg L^{-1} Kin is the optimum and best hormones combination for maximum *in vitro* shoot multiplication of sugarcane genotype Co449 cultures. While MS medium supplemented with 2 mg L^{-1}BAP + 0.5 mg L^{-1}Kin is the best combination for *in vitro* shoot multiplication of sugarcane genotype Co678 cultures.

Effect of BAP and Kin on shoot multiplication of sugarcane *in vitro* cultures on MS medium with table sugar

ANOVA result showed that all the main and interaction effects of genotype, Kin and BAP were very highly significant (p<0.0001) on percentage of shoot induction, average number of shoots per-explants, shoot length and leaves number per shoot (Table 3). Interaction among genotype, Kin and BAP (Gen*Kin*BAP = p < 0.0001) showed interdependence of the three factors for shoot multiplication in the presence of table sugar. The multiplication difference between the two genotypes might be contributed by their difference in level of endogenously accumulated auxins and cytokinins (George and Klerk, 2008).

Among different combinations of BAP and Kin, genotype Co449 showed the highest multiple shoot formation and growth on MS medium supplemented with BAP (2.0 mg L^{-1}) + Kin (0.25 mg L^{-1}) (Table 4) where 80% of the transferred explants produced multiple shoots (7.61 ± 0.10 shoots per explants) that are on an average 6.40 ± 0.10 cm long with 5.33 ± 0.00 leaves per shoot. Increasing concentration of Kin from 0.25 mg L^{-1} to 0.5 mg L^{-1} under the same media composition for the same genotype (Co449) reduced frequency of shoot formation to 73.33% and number of shoots per explant to 6.81. On the other hand, genotype Co678 showed maximum multiplication response on MS medium supplemented with 2 mg L^{-1}BAP + 0.5 mg L^{-1} Kin where 86.67% of the transferred explants gave multiple shoot formation with 8.36 ± 0.04 shoots per explants, 7.27 ± 0.11 cm mean shoot length and 5.56 ± 0.33 leaves per shoot. When lower concentration of Kin (0.25 mg L^{-1}) was used for the same genotype (Co678) on the same media composition, reduced frequency of multiple shoot formation (73.33%) and fewer shoots per explants (7.26 ± 0.13) were achieved.

On the contrary, in both genotypes, the lowest multiplication response (0%) was observed on MS medium devoid of BAP and Kin while shoot multiplication was observed in media containing BAP and Kin (Table 4). This clearly indicates the significance of adding BAP and Kin in tissue culture media for shoot multiplication of sugarcane cultures. Indeed, cytokinins (BAP and Kin) enhance multiple shoots induction by overcoming apical dominancy and releasing lateral buds from dormancy which results in shoot proliferation (Trigiano and Gray, 2005; George and Klerk, 2008). It was also observed that hormone combination that resulted in maximum shoots per explants responded relatively shorter shoot length. This might be the effect of high level of cytokinins that inhibited shoot elongation (George and Klerk, 2008).

Table 4. Effect of BAP and Kin on *in vitro* shoot multiplication of sugarcane cultures on MS medium with table sugar.

Genotype	Hormone combination		Percentage of explants multiplied	Number of Shoots/Explant (Mean ± sd)	Shoot length (cm) (Mean ± SD)	Number of Leaves/Shoot (Mean ± SD)
	Kin (mg/L)	BAP (mg/L)				
	0	0	0.00^{n}	0. 00^{l}± 0.00	0.00^{h} ± 0.15	0.00^{n} ± 0.00
	0	0.5	13.33^{l}	1.33^{j}± 0.00	3.47^{g} ± 0.15	2.33^{kl} ± 0.00
	0	1	20.00^{k}	2.31^{i}± 0.02	4.10^{f} ± 0.10	3.11^{hij} ± 0.19
	0	1.5	53.33^{f}	4.21^{f} ± 0.11	5.17^{d} ± 0.06	3.43^{ghi} ± 0.20
	0	2	73.33^{c}	4.36^{f}± 0.04	6.20^{cb}± 0.20	4.78^{bc}± 0.19
	0.25	0	6.67^{m}	0.67^{k}± 0.58	3.13^{g}± 0.06	1.62^{m} ± 0.11
	0.25	0.5	26.67^{j}	2.57^{i}± 0.06	4.27^{ef}± 0.12	2.89^{ijk} ± 0.19
Co449	0.25	1	40.00^{h}	2.73^{hi}± 0.12	5.23^{d} ± 0.06	3.56^{f-l} ± 0.39
	0.25	1.5	66.67^{d}	5.40^{e} ± 0.10	6.33^{b} ± 0.21	4.21^{c-f} ± 0.18
	0.25	2	80.00^{b}	7.61^{b} ± 0.10	6.40^{b} ± 0.10	5.33^{ab} ± 0.00
	0.5	0	20.00^{k}	2.41^{i}± 0.17	4.30^{ef}± 0.10	1.56^{m} ± 0.02
	0.5	0.5	26.67^{j}	3.23^{gh} ± 0.12	5.43^{d} ± 0.15	2.93^{ijk} ± 0.12
	0.5	1	46.67^{g}	5.36^{e}± 0.04	5.90^{c} ± 0.12	3.83^{d-g} ± 0.29
	0.5	1.5	60.00^{e}	6.38^{d} ± 0.04	6.53^{b} ± 0.06	4.86^{bc} ± 0.23
	0.5	2	73.33^{c}	6.81^{cd}± 0.69	6.37^{b} ± 0.25	5.14^{ab} ± 0.22
	0	0	0.00^{n}	0.00^{l}± 0.00	0.00^{h}± 0.00	0.00^{n} ± 0.00
	0	0.5	13.33^{l}	1.71^{j}± 0.00	3.27^{g}±0.06	2.27^{kl} ± 0.58
	0	1	26.6^{j}	2.44^{i}± 0.00	4.30^{ef}± 0.26	3.42^{ghi} ± 0.21
	0	1.5	40.00^{h}	3.39^{g} ± 0.10	5.13^{d}±0.06	4.33^{cde}± 0.36
	0	2	60.00^{e}	5.56^{e}± 0.19	7.20^{a} ±0.10	4.44^{cd} ± 0.51
	0.25	0	6.67^{m}	2.39^{i}± 0.00	3.20^{g} ±0.10	2.14^{lm} ± 0.17
	0.25	0.5	20.00^{k}	3.78^{fg} ± 0.50	5.30^{d} ±0.36	3.67^{e-h} ± 0.00
Co678	0.25	1	33.33^{i}	4.11^{f} ± 0.08	6.57^{b} ± 0.12	4.42^{cd} ± 0.15
	0.25	1.5	53.33^{f}	4.24^{f} ± 0.77	7.30^{a} ± 0.10	4.44^{cd} ± 0.39
	0.25	2	73.33^{c}	7.26^{bc}± 0.13	7.50^{a} ± 0.20	4.67^{bc} ± 0.58
	0.5	0	20.00^{k}	2.44^{i}± 0.19	4.60^{e} ± 0.10	2.67^{jkl} ± 0.00
	0.5	0.5	46.67^{g}	3.33^{g}± 0.00	5.87^{c} ± 0.12	3.77^{d-h} ± 0.19
	0.5	1	53.33^{f}	5.17^{e}± 0.12	6.27^{cb} ±0.35	4.87^{bc} ± 0.23
	0.5	1.5	60.00^{e}	7.18^{bc} ± 0.14	7.20^{a}± 0.10	5.17^{ab} ± 0.29
	0.5	2	86.67^{a}	8.36^{a} ± 0.04	7.27^{a}± 0.11	5.56^{a} ± 0.33
CV (%)			**5.28**	**5.76**	**3.03**	**6.81**

Means in column with the same letter are not significantly different by Ryan-Einot-Gabriel-Welsch Multiple Range Test α =0.05 significance level.

Similar synergetic effect of BAP and Kin combination on shoot multiplication of sugarcane explants was reported by previous studies. Madhulatha et al*. (*2004) reported that BAP and Kin are generally known to reduce the apical meristem dominance and induce both axillary and adventitious shoots formation from meristematic explants. Geetha and Padmanadhan (2001) reported that the combination of BAP with Kin gave the maximum shoot multiplication response in most sugarcane varieties. Cheema and Hussan (2004) also used the combination of BAP and Kin for the multiplication of six sugar-cane varieties: HSF-240, SPF-213, SPF-234, CP43/33, CP77/400 and CPF237. Ali et al. (2008) observed maximum shoot multiplication response in sugarcane variety BL-4 using the combination of BAP and Kin in MS medium. Khan et al*.* (2009) observed maximum (6-11) shoots per explants in MS medium supplemented with BAP (0.0 - 1.5 mg L^{-1}) and Kin (0.0 - 0.5 mg L^{-1}) in three sugarcane varieties. Khan et al*.* (2006) reported 8.25 ± 0.95 to 11.00 ± 0.81 microshoots per explants using 4% commercial sugar as carbon source in MS media supplemented with BAP.

Hence, on MS medium supplemented with 4% table sugar, BAP (2 mg L^{-1}) + Kin (0.25 mg L^{-1}) was found to be the best combination for shoots multiplication of sugarcane Co449 cultures. While, BAP (2 mg L^{-1}) + Kin (0.5 mg L^{-1}) hormones combination was the best for *in vitro* multiplication of sugarcane genotype Co678 cultures.

The current result indicated that both types of carbon

Table 5. ANOVA summary of effect of IBA and NAA on *in vitro* rooting and root growth on half MS with sucrose.

Source of variation	DF	Mean square		
		Rooting rate	Number of roots/shoot	Root length
Gen	1	96.00***	1.53***	0.29 ***
IBA	4	2357.33***	71.78***	21.75***
NAA	4	2790.68***	20.41***	0.72***
Gen * IBA	4	142.67***	7.02***	0.36***
Gen *NAA	4	302.67***	1.91***	0.23***
IBA * NAA	16	3894.00***	84.42***	22.79***
Gen * IBA *NAA	16	82.67***	2.14***	0.12***
CV (%)		6.89	5.86	5.28

***= Very highly significant (P ≤ 0.0001) at α=0.05 level, DF= Degree of freedom, Gen = Sugarcane genotypes, IBA=Indole-3-butyricacid and NAA=α-naphthaleneacetic acid

source (sucrose or table sugar) showed similar number of shoots per explants and shoot growth comparing Table 2 and 4. Thus, it is possible to deduce that irrespective of the type of carbon source used in the MS medium, 2 mg L^{-1} BAP + 0.25 mg L^{-1} Kin is the optimum hormones combination for multiplication of Co449 cultures and 2 mg L^{-1} BAP + 0.5 mg L^{-1} Kin is the optimum combination for shoot multiplication of genotype Co678 cultures. This result is consistent with the study by Demo et al. (2008) who observed equivalent potato cultures regeneration on MS medium supplemented with table sugar and graded sucrose. The same authors also reported that table sugar not only enhanced micro-propagation but also significantly lowered the production input costs by 34 to 51% when compared with the analytical grade sucrose. Gamborg (2002) and Kodym and Zapata (2001) also reported superior performances of *in vitro* plantlets of banana, chrysanthemum, peanut, and chickpea in medium supplemented with carbohydrates such as glucose, maltose, and table sugar.

Therefore, the present result proved the possibility of utilizing the locally available (in each shop and supermarket), relatively cheap (currently USD 1-1.5 per kg) table sugar as carbon source in place of graded sucrose which is imported and expensive (USD 147 per kg) product in sugarcane tissue culture. Hence, to make developing countries like Ethiopia beneficiary of micropropagation technology, the utilization of such locally available and economically reasonable resources in place of the expensive ones is best alternative.

Effect of IBA and NAA on rooting of *in vitro* raised sugarcane plantlets on half strength MS with sucrose

ANOVA showed very highly significant (p<0.0001) effect of all main and interaction effect of genotype, IBA and NAA (Table 5) on rooting frequency, average roots number per shoot and root length in both genotypes indicating the interdependence of these factors on *in vitro* root induction of sugarcane *in vitro* shoots. The very highly significant effect of genotype indicates existence of genotype difference between the two genotypes on their rooting potential for the same level of IBA and NAA combination: indeed genotype Co449 responded higher rooting frequency than genotype Co678 cultures (Table 6).

Rooting response was not observed in both genotypes on half MS medium devoid of IBA and NAA (Table 6). It was also observed that an increase of IBA from 0.0 to 5 mg L^{-1} maintaining the concentration of NAA at 0.0 mg L^{-1}, increased the rooting frequency of Co449 to 66.67%, with the average roots number per shoot to 7.20 ± 0.10 and average root length to 3.07 ± 0.31 cm. The same trend increased rooting frequency of Co678 cultures to 60%, average roots number per culture to 8.30 ± 0.10 and average roots length to 2.53 ± 0.15cm. In the absence of IBA, an increase of the concentration of NAA from 0.0 mg L^{-1} to 5 mg L^{-1}, increased the rooting frequency in both genotype to 80%, an average roots number per shoot and root length respectively to 12.20 ± 0.52 and 7.10 ± 0.57 cm in Co449 and to 13.17 ± 0.29 and 7.63 ± 0.05 cm in sugarcane genotype Co678 cultures.

Among all combinations of IBA and NAA, genotype Co449 showed the highest rooting frequency (86.67%) with an average roots number per shoot of 16.53 ± 0.02 and root length of 6.90 ± 0.10 cm on half MS medium supplemented with 2.5 mg L^{-1} IBA. When higher concentration of IBA (5 mg L^{-1}) was used for the same genotype (Co449) on the same media composition, decreased frequency of rooting (66.67%), and fewer number of roots per shoot (7.20 ± 0.10) and shorter root length (3.07 ± 0.31 cm) were recorded. On the other hand, genotype Co678 showed the maximum rooting response (80%) with an average roots number per shoot of 13.17 ± 0.29 and root length of 7.63 ± 0.05 cm on half MS medium supplemented with 5 mg L^{-1} NAA. When lower concentration of NAA (2.5 mg L^{-1}) was used for the same genotype (Co678) on the same media composition, reduced frequency of rooting (66.67%) and fewer roots per shoot (8.27 ± 0.11) were observed.

The current result is in agreement with other *in vitro*

Tables 6. ANOVA summary of effect of IBA and NAA on *in vitro* rooting and root growth on half MS with sucrose.

Hormone Combination		Co449			Co678		
IBA (mg/L)	NAA (mg/L)	Explants rooted (%)	Number of Roots Per Shoot	Root length (cm) (Mean ± SD)	Explants rooted (%)	Number of Roots per Shoot	Root length (cm) (Mean ± SD)
0	0	0.00^{m}	$0.00^{u} \pm 0.00$	$0.00^{r} \pm 0.00$	0.00^{m}	$0.00^{u} \pm 0.00$	$0.00^{r} \pm 0.00$
0	1.25	33.33^{hi}	$3.27^{p-s} \pm 0.06$	$1.40^{o-q} \pm 0.10$	40.00^{gh}	$5.27^{j-m} \pm 0.15$	$1.80^{m-o} \pm 0.17$
0	2.5	60.00^{de}	$6.80^{hi} \pm 0.85$	$4.13^{fg} \pm 0.06$	66.67^{cd}	$8.27^{fg} \pm 0.11$	$4.67^{de} \pm 0.15$
0	3.75	73.33^{bc}	$9.83^{e} \pm 0.28$	$5.20^{c} \pm 0.36$	73.33^{bc}	$10.9^{d} \pm 0.28$	$5.27^{c} \pm 0.35$
0	5	80.00^{ab}	$12.20^{c} \pm 0.52$	$7.10^{b} \pm 0.57$	80.00^{ab}	$13.17^{b} \pm 0.29$	$7.63^{a} \pm 0.05$
1.25	0	46.67^{fg}	$3.37^{o-r} \pm 0.06$	$1.53^{n-q} \pm 0.12$	40.00^{gh}	$4.30^{m-p} \pm 0.00$	$1.90^{l-n} \pm 0.00$
1.25	1.25	73.33^{bc}	$6.87^{hi} \pm 0.23$	$2.07^{r} \pm 0.06$	66.67^{cd}	$7.20^{h} \pm 0.72$	$2.57^{k} \pm 0.12$
1.25	2.5	60.00^{de}	$9.07^{ef} \pm 0.12$	$3.17^{ij} \pm 0.29$	60.00^{de}	$7.70^{gh} \pm .26$	$3.57^{h-j} \pm .12$
1.25	3.75	33.33^{hi}	$5.30^{j-m} \pm 0.02$	$4.40^{ef} \pm 0.10$	40.00^{gh}	$5.07^{j-n} \pm 0.67$	$4.53^{ef} \pm 0.15$
1.25	5	20.00^{jk}	$3.50^{o-r} \pm 0.10$	$2.33^{kl} \pm 0.15$	33.33^{hi}	$3.50^{o-r} \pm 0.10$	$2.13^{k-m} \pm 0.06$
2.5	0	86.67^{a}	$16.53^{a} \pm 0.02$	$6.90^{b} \pm 0.10$	73.33^{bc}	$11.37^{cd} \pm 0.55$	$6.77^{c} \pm .25$
2.5	1.25	60.00^{de}	$9.93^{e} \pm 0.62$	$5.30^{c} \pm 0.26$	60.00^{de}	$9.63^{e} \pm 0.35$	$5.53^{l} \pm 0.21$
2.5	2.5	40.00^{gh}	$6.93^{hi} \pm 0.81$	$3.27^{h-j} \pm 0.25$	53.33^{ef}	$6.00^{ij} \pm 0.00$	$3.60^{hi} \pm 0.10$
2.5	3.75	13.33^{kl}	$4.73^{k-n} \pm 0.47$	$3.10^{ij} \pm 0.10$	20.00^{jk}	$4.40^{l-o} \pm 0.10$	$3.27^{h-j} \pm 0.13$
2.5	5	20.00^{jk}	$3.06^{rs} \pm 0.12$	$2.13^{k-m} \pm 0.06$	13.33^{kl}	$3.07^{rs} \pm 0.12$	$2.17^{k-m} \pm 0.06$
3.75	0	73.33^{bc}	$9.90^{e} \pm 0.10$	$5.07^{cd} \pm 0.03$	60.00^{de}	$8.30^{fg} \pm 0.14$	$4.47^{ef} \pm 0.06$
3.75	1.25	46.67^{fg}	$5.37^{j-l} \pm 0.32$	$4.47^{ef} \pm 0.15$	46.67^{fg}	$4.37^{l-o} \pm 0.25$	$3.76^{gh} \pm 0.15$
3.75	2.5	20.00^{jk}	$5.53^{jk} \pm 0.06$	$3.47^{h-j} \pm 0.73$	40.00^{gh}	$3.63^{o-r} \pm 0.15$	$3.47^{h-j} \pm 0.06$
3.75	3.75	13.33^{kl}	$3.50^{o-r} \pm 0.20$	$2.07^{k-m} \pm 0.05$	33.33^{hi}	$3.37^{o-r} \pm 0.06$	$2.13^{k-m} \pm 0.06$
3.75	5	6.67^{lm}	$3.17^{q-s} \pm 0.06$	$1.93^{l-n} \pm 0.07$	20.00^{jk}	$3.13^{q-s} \pm 0.06$	$1.93^{l-n} \pm 0.06$
5	0	66.67^{cd}	$7.20^{h} \pm 0.10$	$3.07^{j} \pm 0.31$	60.00^{de}	$8.30^{fg} \pm 0.10$	$2.53^{k} \pm 0.15$
5	1.25	33.33^{hi}	$4.17^{n-q} \pm 0.57$	$2.33^{kl} \pm 0.15$	26.67^{ij}	$3.57^{o-r} \pm 0.15$	$2.17^{k-m} \pm 0.11$
5	2.5	20.00^{jk}	$3.10^{rs} \pm 0.17$	$2.10^{k-m} \pm 0.10$	20.00^{jk}	$3.20^{q-s} \pm 0.20$	$2.07^{k-m} \pm 0.15$
5	3.75	20.00^{jk}	$1.90^{pq} \pm 0.35$	$1.30^{r} \pm 0.10$	13.33^{kl}	$2.27^{st} \pm 0.15$	$1.76^{m-p} \pm 0.12$
5	5	6.67^{lm}	$1.53^{q} \pm 0.76$	$1.10^{r} \pm 0.10$	6.67^{lm}	$1.67^{t} \pm 0.27$	$1.23^{q} \pm 0.02$
CV (%)		6.89	5.86	5.28	6.89	5.86	5.28

Means in column with the same letter are not significantly different by Ryan-Einot-Gabriel-Welsch Multiple Range Test at α=0.05 significance level.

root induction reports of sugarcane cultures. Bekesha et al. (2002) observed 85% rooting response with an average number of roots per shoot of 15 ± 0.5 and an average root length of 4 ± 0.5 cm on half MS supplemented with 5 mg L^{-1} NAA. Behara and Sahoo (2009) observed 85% *in vitro* rooting on half MS + 2.5 mg L^{-1} NAA with average number of roots per microshoot of 11 ± 1.5, and average length of roots (cm) 4.0 ± 0.94. Alam et al. (2003) reported best rooting response at 2.5 mg L^{-1} IBA with 16 numbers of roots per explants having 1.1 cm root length. Mamun et al. (2004) obtained best results of rooting on MS medium supplemented with auxins (NAA + IBA) 0.5 mg L^{-1} for each one. However, the current result is not in accordance with the report of Ali et al. (2008) who reported 100% rooting on medium containing 1.0 mg L^{-1} NAA and 2.0 mg L^{-1} IBA with 2.8 roots per shoot in sugarcane variety CP 77,400 and 3.1 roots per shoot in variety BL-4.

Therefore, half MS medium supplemented with 2.5 mg L^{-1} IBA + 6% sucrose was the optimum combination for rooting of *in vitro* multiplied shoots of sugarcane genotype Co449 while half MS + 5 mg L^{-1} NAA + 6% sucrose was found to be the optimum medium combination for *in vitro* rooting of shoots of genotype Co678.

Effect of IBA and NAA on rooting of *in vitro* raised sugarcane plantlets on half strength MS with table sugar

ANOVA showed that genotype, IBA, NAA and their interactions had very high significant ($p<0.0001$) effects on rooting frequency, average root number per shoot and root length (Table 7). Interaction of genotype by IBA by NAA indicated that all the three factors are dependent on each other in influencing *in vitro* rooting of sugarcane cultures in the presence of table sugar. Of the two genotypes, genotype Co449 showed higher rooting frequency

Table 7. ANOVA summary of effect of IBA and NAA on *in vitro* rooting and root growth on half MS with table sugar.

Source of variation	DF	Mean square		
		Rooting rate	Number of roots/shoot	Root length
Gen	1	130.67***	0.82***	0.26***
IBA	4	2644.00***	71.47***	20.56***
NAA	4	3210.68***	23.81***	0.62***
Gen* IBA	4	204.00***	3.77***	0.27***
Gen* NAA	4	197.34***	0.90**	0.32***
IBA* NAA	16	3847.34***	86.24***	22.92***
Gen* IBA *NAA	16	104.00***	1.69***	0.15***
CV (%)		4.04	7.23	5.53

***= Very highly significant (P ≤ 0.0001) at α=0.05 significance level **= highly significant (P= 0.0009) at p<0.05 DF= Degree of freedom, Gen = Sugarcane genotypes, IBA=Indole-3-butyricacid and NAA=α-naphthaleneacetic acid.

than genotype Co678 (Table 8) and the variation might be due to their difference in the level of endogenously accumulated PGRs.

In both genotypes, no rooting response was recorded on half MS media devoid of IBA and NAA and rooting occurred in media supplemented with IBA and/or NAA indicating the significance of adding auxin/s in root induction media for rooting of *in vitro* generated sugarcane microshoots (Table 8). Among the different treatment combinations, genotype Co449 showed the highest rooting frequency (86.67%) with an average root number per microshoot of 15.93 ± 0.81 and root length of 7.17 ± 0.15 cm on half MS with 2.5 mg L^{-1} IBA. However, for the same genotype (Co449), an increase in concentration of IBA (5 mg L^{-1}) maintaining the concentration of NAA at 0.0 mg L^{-1} resulted in significantly reduced rooting frequency (60%), lesser number of roots per shoot (7.10 ± 0.10) and shorter root length (3.07 ± 0.12 cm). On the other hand, genotype Co678 showed the maximum root number per microshoot and root length on half MS medium supplemented with 5 mg L^{-1} NAA. On this medium, 80% of the transferred microshoots of genotype Co678 produced the highest root number (13.93 ± 0.81) per microshoot with an average root length of 7.33 ± 0.64 cm. Half MS + 2.5 mg L^{-1} IBA which resulted best rooting in genotype Co449, gave 80% rooting frequency with an average of 12.17 ± 0.76 roots per microshoot and root length of 6.57 ± 0.12 cm in genotype Co678, thus this medium can be taken as the second best medium combination for rooting of this genotype.

The current result agrees with other *in vitro* root induction reports on sugarcane. Khan et al. (2006) stated that the types and concentrations of auxin/s in the rooting media influence the root induction response of sugarcane cultures. Gopitha et al. (2010) observed 80% rooting frequency with mean roots number per microshoot of 9.6 and root length of 3.9 cm on half MS medium supplemented with 5 mg L^{-1} NAA. The same authors reported 54% rooting frequency with an average of 8.8 roots per microshoot and 3.8 cm root length on half MS medium containing 7 mg L^{-1} NAA indicating higher concentration of auxin(s) result in reduced rooting responses (rooting frequency, root number and root length). Singh (2003) reported the highest rooting frequency (85%) with an average of 13.33 ± 0.6 roots per shoot and root length of 3.8 cm using 5 mg L^{-1} NAA. Many workers also reported that 5 mg L^{-1} NAA was good for rooting of sugarcane microshoots (Shukla et al., 1995; Islam et al., 1996; Gosal et al., 1998; Lal et al., 2001) and more than 5 mg L^{-1} NAA inhibits rooting. Alam et al. (2003) reported best rooting at 2.5 mg L^{-1} IBA with 16 roots per explants having 1.1 cm root length.

On the tops of the current result, it is fair to deduce that half strength MS + 2.5 mg L^{-1} IBA + 6% table sugar is the optimum combination for maximum rooting of *in vitro* generated microshoots of sugarcane genotype Co449 cultures. Whereas, half strength MS + 5 mg L^{-1} NAA + 6% table sugar is found to be the best combination for *in vitro* rooting of Co678 shoots. As can be verified from Tables 6 and 8, for a given genotype and for a given level of IBA and NAA combination, rooting responses on both carbon sources were not significantly different. Demo et al. (2008) also observed an equivalent number of roots per shoot on graded sucrose (7.2) and table sugar (7.5) supplemented media. Thus, it is fair to deduce that table sugar which is locally available and affordable can be used as an alternative carbon source in rooting media also.

Acclimatization of plantlets

For the two genotypes, different acclimatization potential was observed: 80% for genotype Co449 and 86.67% for genotype Co678. Loss of some plantlets might be due to the variation in the method of propagation and environmental factors: Temperature and humidity. The less development of cuticle under *in-vitro* condition and the drop in relative humidity from near 100% in the culture vessels

Table 8. Effect of IBA and NAA on root induction of *in vitro* raised sugarcane plantlets on half strength MS with 6% table sugar.

Hormone combination		Co449			Co678		
IBA (mg/L)	NAA (mg/L)	Explants rooted (%)	Number of Roots Per Shoot	Root length (cm) (Mean ± SD)	Explants rooted (%)	Number of Roots per Shoot	Root length (cm) (Mean ± SD)
0	0	0.00^{m}	$0.00^{u} \pm 0.00$	$0.00^{r} \pm 0.00$	0.00^{m}	$0.00^{u} \pm 0.00$	$0.00^{r} \pm 0.00$
0	1.25	33.33^{i}	$3.20^{o\text{-}r} \pm 0.10$	$1.47^{opq} \pm 0.15$	46.67^{g}	$5.27^{lm} \pm 0.15$	$1.87^{no} \pm 0.06$
0	2.5	53.33^{f}	$6.70^{ij} \pm 0.82$	$3.73^{g} \pm 0.29$	6.00^{e}	$8.27^{efg} \pm 0.11$	$4.77^{de} \pm 0.06$
0	3.75	60.00^{e}	$9.43^{de} \pm 0.51$	$5.13^{cd} \pm 0.32$	73.33^{c}	$10.37^{d} \pm 0.21$	$5.27^{cd} \pm 0.35$
0	5	80.00^{b}	$12.33^{c} \pm 0.76$	$7.50^{a} \pm 0.26$	80.00^{b}	$13.93^{b} \pm 0.81$	$7.33^{a} \pm 0.64$
1.25	0	46.67^{g}	$3.40^{n\text{-}q} \pm 0.10$	$1.33^{pq} \pm 0.21$	40.00^{h}	$4.30^{m\text{-}p} \pm 0.00$	$2.00^{mno} \pm 0.10$
1.25	1.25	73.33^{c}	$6.53^{ijk} \pm 0.50$	$2.13^{lmn} \pm 0.15$	60.00^{m}	$7.20^{ghi} \pm 0.72$	$2.67^{jkl} \pm 0.06$
1.25	2.5	60.00^{e}	$9.50^{d} \pm 0.10$	$3.20^{ghi} \pm 0.26$	6.00^{e}	$7.70^{ghi} \pm 0.26$	$3.57^{gh} \pm 0.12$
1.25	3.75	33.33^{i}	$5.23^{lm} \pm 0.25$	$4.53^{ef} \pm 0.15$	40.00^{h}	$5.10^{lm} \pm 0.69$	$4.60^{ef} \pm 0.26$
1.25	5	20.00^{j}	$3.50^{n\text{-}q} \pm 0.10$	$2.47^{kml} \pm 0.15$	33.33^{i}	$3.50^{n\text{-}q} \pm 0.10$	$2.20^{lmn} \pm 0.10$
2.5	0	86.67^{a}	$15.93^{a} \pm 0.81$	$7.17^{a} \pm 0.15$	80.00^{b}	$12.17^{c} \pm 0.76$	$6.57^{b} \pm 0.12$
2.5	1.25	66.67^{d}	$9.60^{d} \pm 0.79$	$5.30^{c} \pm 0.26$	6.00^{e}	$9.80^{d} \pm 0.62$	$5.57^{b} \pm 0.25$
2.5	2.5	40.00^{h}	$6.93^{hi} \pm 0.81$	$3.33^{ghi} \pm 0.15$	46.67^{g}	$6.93^{l\text{-}o} \pm 0.81$	$3.660^{gh} \pm 0.10$
2.5	3.75	13.33^{k}	$4.56^{lmn} \pm 0.31$	$3.10^{hij} \pm 0.10$	20.00^{j}	$4.40^{k} \pm 0.10$	$3.\ 33^{ghi} \pm 0.12$
2.5	5	20.00^{j}	$2.93^{qrs} \pm 0.32$	$2.20^{lmn} \pm 0.10$	13.33^{k}	$3.00^{p\text{-}s} \pm 0.20$	$2.23^{lmn} \pm 0.06$
3.75	0	73.33^{c}	$9.27^{def} \pm 0.23$	$5.13^{cd} \pm 0.06$	66.67^{d}	$9.33^{def} \pm 0.12$	$4.47^{ef} \pm 0.06$
3.75	1.25	46.67^{g}	$5.23^{l} \pm 3\ 0.32$	$4.47^{ef} \pm 0.15$	$46.67^{g}4$	$5.37^{klm} \pm 0.25$	$4.23^{f} \pm 0.12$
3.75	2.5	20.00^{j}	$5.37^{klm} \pm 0.32$	$3.47^{gh} \pm 0.06$	40.00^{h}	$5.63^{jkl} \pm 0.15$	$3.47^{gh} \pm 0.06$
3.75	3.75	13.33^{k}	$3.50^{n\text{-}q} \pm 0.20$	$2.10^{mn} \pm 0.00$	3.33^{i}	$3.50^{n\text{-}q} \pm 0.20$	$2.20^{lmn} \pm 0.10$
3.75	5	6.67^{l}	$3.07^{p\text{-}s} \pm 0.06$	$1.93^{mno} \pm 0.06$	13.33^{k}	$3.13^{o\text{-}s} \pm 0.06$	$1.93^{mno} \pm 0.06$
5	0	60.00^{e}	$7.10^{ghi} \pm 0.10$	$3.07^{hij} \pm 0.12$	66.67^{d}	$8.16^{fgh} \pm 0.06$	$2.90^{ijk} \pm 0.26$
5	1.25	33.33^{i}	$4.16^{n\text{-}q} \pm 0.56$	$2.17^{lmn} \pm 0.16$	20.00^{j}	$3.50^{n\text{-}q} \pm 0.10$	$2.20^{lmn} \pm 0.10$
5	2.5	20.00^{j}	$3.17^{o\text{-}s} \pm 0.15$	$2.07^{mn} \pm 0.06$	13.33^{k}	$3.17^{o\text{-}s} \pm 0.15$	$2.10^{mn} \pm 0.10$
5	3.75	20.00^{j}	$1.90^{st} \pm 0.35$	$1.80^{nop} \pm 0.10$	13.33^{k}	$1.90^{st} \pm 0.35$	$1.93^{mno} \pm 0.06$
5	5	6.67^{l}	$1.40^{t} \pm 0.53$	$1.27^{q} \pm 0.21$	6.67^{l}	$1.03^{rs} \pm 0.06$	$1.17^{q} \pm 0.12$
CV (%)		**4.04**	**7.23**	**5.53**	**4.04**	**7.23**	**5.53**

Means in column with the same letter are not significantly different by Ryan-Einot-Gabriel-Welsch Multiple Range Test at α=0.05 significance level.

to much lower values in the poly house might result in excessive water loss and death (Biradar et al., 2009). The current result is in agreement with the report of Ali et al. (2008) who declared 70-80% greenhouse acclimatization potential of *in vitro* generated sugarcane cultures. Biradar et al. (2009) also reported 72% survival rate of micropropagated plantlets.

Conflict of Interests

The author(s) have not declared any conflict of interests.

Conclusion

Lack of a steady supply of good planting material is one of the bottle necks for the exploration of the potential of sugarcane in Ethiopia. Mass propagation of sugarcane through shoot tip culture ensures quick availability of genetically uniform (true to type) diseases free planting materials within short period of time. In the present study, an effective protocol for subsequent *in vitro* plantlets multiplication from shoot tip explants was developed for sugarcane genotypes Co449 and Co678. Accordingly, irrespective of the type of carbon source (3% sucrose or 4% table sugar) used in the MS medium, the combination of 2 mg L^{-1} BAP + 0.25 mg L^{-1} Kin is found to be the best combination for shoot multiplication of genotype Co449 while 2 mg L^{-1} BAP + 0.5 mg L^{-1} Kin is the optimum combination for shoot multiplication of genotype Co678. Regarding *in vitro* rooting, half MS medium + 2.5 mg L^{-1} IBA is found to be the best combination for rooting of microshoots of genotype Co449 while half MS + 5 mg L^{-1} NAA is the best media combination for maximum rooting of microshoots of genotypes Co678. The study also revealed that shoot multiplication and rooting responses on sucrose or table sugar supplemented media are not significantly different. Hence, it is fair to deduce that we have developed a cost effective protocol, which can use table sugar instead of costly graded sucrose for *in vitro*

mass propagation of two commercial sugarcane genotypes grown in Ethiopia: Co449 and Co678. Hence, to make developing countries like Ethiopia beneficiary of micropropagation technology, the utilization of such locally available and economically reasonable resources in place of the expensive ones is best alternative.

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