Advances in Crop Science and Technology

ISSN: 2329-8863

Olani G,Shibiru S,Debebe A, Adv Crop Sci Tech
2021, 9:10

Research Article Open Access

Micro Propagation Protocols for Five Sugarcane Cultivars

Gamachu Olani* ,**Surafel Shibiru, Abel Debebe**

Department of Agriculture, Melkassa Agricultural Research Center (MARC), Ethiopia

***Corresponding author:** Gamachu Olani, Department of Agriculture, Melkassa Agricultural Research Center (MARC), Ethiopia, Email: gamachuolani@gmail.com*

Received date: November 02, 2021; **Accepted date:** November 16, 2021; **Published date:** November 23, 2021

Citation: Olani G, Micro Propagation Protocols for Five Sugarcane Cultivars , Adv Crop Sci Tech. 9: 486

Abstract

In vitro propagation protocol optimized for five Sugarcane cultivars, namely V2-111, V2-333, V2-444, V2- 555 and V2-999 studied. Stems sterilized with 2% local bleach for 20 minutes within laminar flow before their culture on hormone free basal media for their establishment. MS media with 1mg/L BAP+0.1mg/L GA3 resulted a significant number shoots for cultivars V2-333; and 0.5mg/LBAP+0.5mg/L KN, 0.5mg/LBAP alone and hormone free for V2-999, V2-111 and V2-555 with mean of 10.5, 16.3 and 28.2 shoots respectively. All treatments except hormone free resulted better numbers for V2-444, that is 25.2. Finally, when shoots transferred for their rooting and elongation, 1/2MS supplemented with 3mg/LNAA and 1.5mg/LIBA resulted best numbers of roots across all cultivars with means 5.45 and 5.00 respectively. After 30 days, rooted plantlets acclimatized in maintained greenhouse resulting highest 100% survival for cv V2-555.

Keywords: MS; Sugarcane; In vitro; micro propagation; BAP; IAA

Introduction

Sugarcane, a crop of family Poaceae genus Saccharum, is a crops widely cultivated around tropics and sub-tropical countries for its sugar production. While sugarcane was first dedicated to sugar production, advances in technology have ensured that nowadays, all parts of the sugarcane plant can be converted into energy [1]. To date, producing bioethanol from the sugar in sugarcane (first-generation biofuels) has been one of the world's most commercially successful biofuel production systems. Sugarcane is almost a vegetatively propagated crop by nodal cuttings, though they had a disadvantages, viz. slow multiplication and disease transmission. In these regards, so far sugarcane in vitro culture has been utilized for micropropagation of genotypes, production of disease-free material from excised apical meristems, international germplasm exchange, generation of somaclones, rapid disease and pest resistance screening and germplasm conservation. In Ethiopia, apart from its production for sugar productions, it was also cultivated locally in small plots for its juice consumption locally from its sets. According to the information from Ethiopian Sugar Corporation, about 400,000MT (2015/16) sugar produced annually and the country is under the construction of additional 6 factories in addition to previously functional 4 factories to become one of the ten largest sugar producers by 2023 (USDA, 2015). For this purpose, the corporation brought better varieties from abroad and along with conventional means aimed to propagate them in vitro through plant tissue culture techniques. Plant biotechnology via its micro propagation had been used to propagate large quantities of clean plants using either apical meristems or organs in small areas within a short periods of time and the technology introduce to the country since 1980. Different scholars reported micro propagation techniques using shoot tips and somatic embryogenic routes. Although *in vitro* sugarcanes had well-established protocols done with different scientists, their genotypic response may vary in culture, requiring refinement of plant growth regulator concentration and ratios for improved propagation efficiency. Hence, the present study intended to optimize in vitro micro propagations of five sugarcane cultivars [2].

Materials and Methods

Sterilization of the cultivars

Five elite commercial varieties, namely V2-111, V2-333, V2-444, V2-555 and V2-999, from Wonji Sugar factory were used to optimize the multiplication protocol. The cultivars were brought from Werer Agricultural Research center were they varieties are kept under quarantine conditions, and stem cuttings with two buds from each were brought and planted on pot planted in our screen house facilities as a mother plant for the experiment at Melkassa Agricultural Research Center. Once they reached 30cm long, shoot tips excised from the stem taken to plant tissue culture laboratory. Washed with detergents and brush, treated by 2% local bleach (locally called Barekina) for 20min before its size reduced down to about 4cm to its inner shoots. The shoots then finally sterilized again by 1.5% barekina within laminar hood using sterilized distilled water for another 15min, and washed thoroughly three times with the same distilled water (Figure 1).

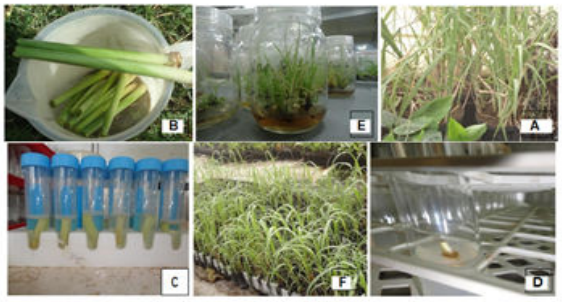

Figure1: Procedural pictures followed for Sugarcane in vitro culture (A: Sugarcanes planted within greenhouse on seedling pots (Mother plant preparation), B: Excised shoots tips of app. 15cm from their Mother Plant, C: Trimmed down and under sterilization in the laboratory; D: Initiated sugarcane, E: Sugarcanes plantlets on their multiplication treatments, and F: Acclimatized seedlings with in green house).

Basal/Nutrient Media

The initiation of all varieties was done on Murashige and Skoog (MS) media devoid of hormone and containing only 0.1gm/L thiamine. After the initiation of explants, plantlets were multiplied on low cytokinin containing media (0.5 mg/L) being refreshed every four weeks, in order to obtain enough amounts of explants to start the optimization of multiplication media. During multiplication experiment MS media with five different combinations; that is 0.5mg/L BAP and 0.5 mg/L Kinetin (1), hormone free (2), 1mg/L BAP alone, 1mg/L BAP and 0.1mg/L GA3 (4) and 0.5mg/L BAP alone (5) were used. Finally, for rooting we used a media with half MS media containing seven different hormone combination which includes: hormone free, 0.5mg/L NAA, 3mg/L NAA, 5mg/L NAA, 0.5mg/L IBA, 1.5mg/L IBA, and 3mg/L IBA as well as full MS with hormone free (8) and 2mg/L NAA alone [3]. Acclimatization of the seedlings rooted were done in green house on sterilized soil mix composed of filter cake and sand mixed together in 3:1.

Data collection and statistical analysis

The experiment designed in CRD with five and nine hormone combinations treatment (for multiplication and rooting respectively) each being replicated five for multiplication and six times for rooting. Four explant per jar cultured throughout the experiment. Data's like the numbers of shoots and their multiplication factors while refreshed to new media, and weekly numbers of rooted explants for consecutive six weeks during rooting stage, finally weekly numbers of survivals out of the total shoots acclimatized during their initial five weeks in green house. Numbers of shoots and multiplication factors were log(x +1) transformed and analyzed by Statistix 8 software their ANOVA and mean averages.

Result and Discussion

Establishment and multiplication

Before their establishment in laboratory, shoots 30cm long excised from sugarcane stems grown in green house and sterilized. Washed with detergents and brush, treated by 2% local bleach (locally called Barekina) for 20min before their size trimmed down to about 4cm to its inner shoots. Finally, sterilized again by 1.5% barekina within laminar hood using sterilized distilled water for another 15min, and washed thoroughly three times with the same distilled water before their transfer on Basal media devoid of hormone and containing only 0.1gm/L thiamine for initiation. After the initiation of explants, plantlets were multiplied on low cytokinin containing media (0.5 mg/L) until enough amounts of explants to start multiplication screening obtained. Usually Cytokinins like BAP, BA or Kinetin alone or along with small amount of auxin used for shoot proliferation. (**Table 1**). Different varieties have shown different reaction to the multiplication treatments used based on the statistical analysis.

Source	DF	SS	MS	F	P
TRT	4	6.4847	1.62118	55.32	0
VARIETY	4	7.6203	1.90507	65	0
TRT*VARIETY	16	7.3856	0.4616	15.75	0
Error	225	6.5942	0.02931		
Total	249	28.0848			

Table1: Analysis of Variance for means of number of shoots.

Based on mean separation all treatments indicated significant difference with their mean number of shoots across all varieties except treatment 3 with 1mg/L BAP alone (Error! Not a valid bookmark self-reference.). MS media devoid of any hormone resulted best mean number of shoots for V2-555 and all treatments included except hormone free indicated similar means in V2-444. Treatment 5, 0.5mg/l BAP alone, and 0.5mg/L BAP along with 0.5mg/L kinetin gave means of 16.3 and 10.5 for V2-111 and V2- 999 varieties respectively. In case of variety V2-333, hormonal treatment with 0.5mg/L BAP &0.5MG/L KN and the one with 1mg/L BAP & 0.1mg/L GA3 showed 22.3 & 22.7 mean numbers of shoots. Unlike others cultivar v2-555 on hormone free gave significant number of shoots when compared with other (mean=20.14) [5]. This multiplication stage in fact is crucial in propagation of any crop species for commercial purpose and most rapid rates are required also reported the optimum multiplication for var. HSF- 240, CP-77-400 and CPF-237 at 1.5mg/l BAP with 0.5mg/l Kin, 1.0 mg/l BAP with 0.5mg/l Kin, and 1.0mg/l BAP with 0.1mg/l Kin. In addition Ali et al. (Ali et al., 2008) obtained maximum shoot multiplication in CP 77400 and BL-4, found 29 shoots on MS medium with 1.0mg/l BAP, and 0.25mg/l BAP and Kin respectively also obtained best shoots for Isd-28 and Isd-29 on MS medium fortified with 1.5mg/l BA and 0.5mg/l NAA. In this study, 1mg/L BAP and 0.1mg/L GA3 gave better numbers of shoots for cv V2-333 and media devoid of hormone gave significant number of shoots for cv V2-555.

Treatments	Sugarcane varieties									
MS	V2-555		V2-111		V2-999		V2-333		V2-444	
	Mean		Mean	Comp.	Mean		Mean	Comp.	Mean	Comp.

T1	0.5 mg/L BAP and 0.5 mg/L Kinetin	23.4	AB	12.1	AB	10.5	A	22.3	A	24.7	A
T2	hormone free	28.2	A	5.8	B	3.8	B	3.7	B	2	B
T3	1mg/L BAP alone	26.7	A	6.1	B	7	AB	15.2	AB	16.1	A
T4	1mg/L BAP and 0.1 mg/L GA3	11.9	B	14.6	A	5.4	AB	22.7	A	20.5	A
T5	0.5 mg/L BAP alone	14.4	AB	16.3	A	7.2	AB	15.1	AB	25.2	A

Table2: The mean numbers of shoots across the shoot multiplication treatments.

Rooting and acclimatization

The final success of in vitro propagation relies on efficient rooting in regenerated shoot and their subsequent acclimatization. Both full and half strength Murashige and Skoog basal media were used as late rooting treatments along with auxin hormones, specifically NAA and IBA. Across all varieties ½ MS media having 3mg/L NAA and 1.5mg/L IBA resulted grand mean number of 5.45 and 5.00 roots respectively (**Table 3**). Khan et al. (Khan et al., 2006) reported also vigorous root development on MS medium containing 6% table sucrose +1mg/l IBA among the combinations used. However, unlike in the present study, the MS medium without growth regulators promoted rooting in more than 90% for other two sugarcane cultivars after 30 days of culture (Dibax et al., 2011; Singh et al., 2006). Jagadeesh et al. (Jagadeesh, B., Kumar, M., Shekhar, M. & Udhakar, 2011) on another hand reported NAA as a better for rooting of sugarcane than IBA either alone or in combination with other hormones. In general, but many researchers reported that 5 mg/l NAA was good for rooting (R. Baksha, R.Alam, M.Z. Karim, S.K. Paul, M.A. Hossain, 2002; Yadav, S., Ahmad, A. & Lal, 2012) [16,65]. A concentration exceeding 5mg/l NAA inhibits rooting. In contrary, many researchers obtained best rooting at lower concentration of NAA from 0.5-3mg/l. Unlike in other crops where media devoid of any hormone results significant numbers in numbers of roots and acclimatization, in current studies lowest numbers were registered during both scenario's for all varieties. However, Dibax et al., (Dibax et al., 2011) and Singh, 2006 reported 90% promoted roots for two cultivars; cvRB931003 and RB98710 via embryo development. In addition to the hormones utilized, rooting of a sugarcane shoots greatly depends on Basal media strength. reported that half MS media were more responsive than full MS medium for rooting of sugarcane. Reducing the strength of basal medium by half also aids in lowering osmotic pressure within media and favors root formation in sugarcane like during their *in vivo* states.

Treatments		**Sugarcane varieties**									
Media formulation		**V2-555**		**V2-111**	**V2-999**	**V2-333**	**V2-444**				
		Mean	**Mean Comp.**	**Mean**	**Mean Comp.**	**Mean**	**Mean Comp.**	**Mean**	**Mean Comp.**	**Mean**	**Mean Comp.**
T1	1/2 MS alone	4.81	A	3.88	ABC	3.03	A	3.04	A	5.78	AB
T2	1/2 MS +0.5 mg/L NAA	5	A	4.87	AB	3.44	A	4.34	A	6	A
T3	1/2 MS +3mg/L NAA	4.81	A	6.43	A	5	A	5	A	6	A
T4	1/2 MS +5mg/L NAA	4.81	A	7	A	4.56	A	3.84	A	5.3	AB
T5	1/2 MS +0.5 mg/L IBA	4.46	AB	1.82	BC	1.14	B	3.14	A	5.2	B
T6	1/2 MS +1.5 mg/L IBA	4.81	A	6.78	A	3.48	A	3.92	A	6	A
T7	1/2 MS +3mg/L IBA	4.81	A	5.6	AB	2.41	A	4.46	A	6	A
T8	MS alone	3.87	B	1.62	C	1.01	B	1.09	B	5.37	AB
T9	MS +2mg/L NAA	4.3	AB	4.53	AB	3.29	A	2.51	A	6	A

Table3: Mean number of roots and their mean separation.

TRT	Media formulation	Sugarcane varieties/ Mean Surv				
		V555	V111	V333	V999	V444
1	1/2MS alone	81.8	41.1	12.9	9.4	17.2
2	1/2MS +0.5mg/L NAA	100	19.3	13.8	15.3	24.7
3	1/2MS +3mg/L NAA	98.5	47.3	30.1	41.9	32.7
4	1/2MS +5mg/L NAA	100	31.4	37.8	14.5	23
5	1/2MS +0.5mg/L IBA	97.1	47.5	12.3	24.2	16.6
6	1/2MS +1.5mg/L IBA	79.7	24.3	23.1	12.8	18.5
7	1/2MS +3mg/L IBA	100	61.8	2.6	10.4	15.8
8	MS alone	79.3	26.9	4.4	3	4.5
9	MS +2mg/L NAA	84.8	41.9	1.3	10.9	1.3

Table4: Mean Percentage of survived plantlets during Acclimatization after fifth week.

In Banana, a change in media from cytokinin to hormone free or waiting on the same media for more than a month also generates roots that can enables their acclimatization in green house (Dagnew et al., 2012). And Surafel et al. also reported Aloe vera cultured devoid of hormones generating good roots (Surafel et al., 2018). The difference with regard to these may be due to the more heterogeneous nature of sugarcane and their response in each stage too varies widely for hormones between one another than other crop species. Finally, plantlets rooted were taken out for their acclimatization in green house within maintained temperature and humidity. Before they were planted, a combination of filter cake and soil in 3:1 ratio sieved and sterilized by dry oven. Highest percentage of survival (100%) registered for cultivar V2-555. In general, since a tissue culture technology is a tool for mainly commercial production of plantlets, its utilization through a cost minimized way recommended. In this regard rather than using costly gelling agents, using liquid and temporary immersion (RITA system) media was preferred even for efficiency of propagating sugarcane in large quantities.

Acknowledgement

We would like to thank ESC and EIAR for their financial support and other whenever required. We are thankful for our staff members in Melkassa Agricultural Research Center, who enable this study at different position.

References

1. Ben-Iwo J, Manovic V, Longhurst P (2016). Biomass resources and biofuels potential for the production of transportation fuels in Nigeria. Renew Sustain Energy Rev 63: 172–192.
2. Dibax R, Alcântara GB de, Filho JCB, Machado MP, Oliveira Y, et al. (2011) Plant regeneration of sugarcane cv. RB931003 and RB98710 from somatic embryos and acclimatization. J Biotechnol Biodiv 2: 32–37.
3. Hailu M (2017) In Vitro Propagation of Selected Sugarcane (Saccharum officinarum L.) Varieties (C 86-56 and C 90-501) through Apical Meristem. Adv crop sci 5.
4. Mulugeta Diro BT (2014) In vitro Aseptic Culture Establishment of Sugarcane (Saccharum officinarum L.) Varieties Using Shoot Tip Explants. Adv crop Sci Technol 02: 2–7.
5. Mamun MA, Sikdar MB, Paul DH, Rahman M, Rezuanul Islam M, (2004). In vitro micropropagation of some important sugarcane varieties of Bangladesh Asian J Plant Sci 3: 666–669.