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Interaction Effects of 6-Benzylaminopurine and Kinetin on *In vitro* Shoot Multiplication of Two Sugarcane (*Saccharum officinarum L.*) Genotypes

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Abstract

In Ethiopia, sugarcane is grown as an important cash and industrial crop. It is not an ideal crop for conventional breeding and it lacks rapid multiplication procedures to commercialize newly released varieties within a short period of time. Hence, the objective of this work was to optimize the optimum concentration of 6-benzylaminopurine (0.0, 0. 5, 1.0, 1.5, 2.0, 2.5, and 3.0 mgL-l) and Kinetin (0.0, 0.1, 0.5, and 1.0 mgL-l) combination for shoot multiplication of C86-12 and C86-56 genotypes in completely randomized design with 5x4x2 factorial treatment combinations arrangements. The analysis of variance showed that the interaction effects of BAP, kinetin and genotypes on the number of shoots per explant, number of leaves per shoot and average shoot length were highly significant ($p<0.001$). Murashige and Skoog (MS) medium supplemented with 1.5 mgL-l BAP and 0.5 mgL-1 of Kin for B86-12; and 1.5 mgL-1 of BAP and 1.0 mgL-1 of Kin for C86-56 were found to be the optimum media for shoot multiplication. B86-12 showed 33.8 ± 0.837 number of shoots per explant with 13.04 ± 0.089 average number of leaves per shoot and 8.4 ± 0.008 cm shoot length whereas C86-56; 25.6 ± 0.548 number of shoots per explant with average number of leaves per shoot of 9.8 ± 0.447 and shoot length 8.65 ± 0.72 cm was obtained after 30 days of sub culturing. Thus, the optimized protocol can be used for rapid multiplication of the planting materials for commercializing the newly released genotypes within a short period of time.

Keywords: Sugarcane; BAP; Kin; Shoot multiplication

Introduction

Sugarcane (*Saccharium officinarum L.*) belongs to the Saccharium genus of the Andropogoneae tribe of the Poaceae (Gramineae/grass) family with an octaploid 2n=8x=80 number of chromosomes [1]. It is a tall perennial tropical grass that tillers at the base to produce unbranched stems, 3-4 m or more in height with a thickness of approximately 5 cm in diameter. It is one of the most efficient photosynthesizing plant or converter of solar energy to sugar stored in the internode [2]. The commercially cultivated crops of sugarcane have two geographic centers of origin in New Guinea and Northern India [3]. Although the major industries are found in Brazil, China and India, the crop is also commercially produced in many other countries, including South Africa [4]. But there is no well documented reference on how, where and when sugarcane was introduced to Ethiopia, although some records claim its introduction during the early 18th century [5].

Sugarcane accounts approximately 75% of the world's sugar and it is economically important cash crop in tropical and sub-tropical regions of many countries [6]. Its properties such as efficient photosynthesis and biomass production make it an excellent target for industrial processing, valuable alternative for animal feed and for the production of by-product such as ethanol from molasses [7]. In Ethiopia, this crop is grown as an important cash and industrial crop among many crops and it has an immense importance for the development of the economy of the country. It is used for the production of white and brown sugar and by-products like molasses, bagasse and press mud (filter cake) which have been used for different purposes in a daily life and there is no by-product thrown as non- useful matter. Furthermore, production of sugar in Ethiopia has created employment opportunities and foreign currency.

Day by a day increasing use of sugar and its relevant by-products has created a challenging situation for sugar producing countries, researchers and growers [8]. In Ethiopia the annual yield of sugar from three factories was nearly around 300,000 tons while the annual domestic demand is close to 450,000 tons [9] and the deficit was covered by importing from abroad. In addition, the yield per hectare of this crop is the lowest all over the world [8]. Considering the availability of abundant water resource coupled with a vast fertile land favorable for sugarcane cultivation, suitable agro-ecological conditions, cheap labor and huge domestic and foreign demand for sugar and for its by-products [9], this yield is very minimal in the country. Hence, by considering these opportunities the government has planned to establish ten sugar factories on 370,000 ha of plantation area.

Yield potential of sugarcane varieties is deteriorating day by day due to segregation, susceptibility to diseases, insects, admixture, and changes in edaphic and climatic factors [10]. Improvement of sugarcane varieties is very difficult, because it is not an ideal candidate crop for conventional plant breeding, since its flowering is not-synchronized, it has low sexual seed viability and it is a perennial crop [11]. Hence, its improvement takes up to ten years from initial crosses to final agronomic assessments [12,3]. But also the lack of rapid multiplication

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procedure has long been a serious problem in sugarcane conventional breeding programs as it takes 10-15 years of work to complete a selection cycle [13,8]. Commercially, sugarcane is propagated from stem cutting with each cutting or set having two or three buds [14,15]. Vegetative propagation by cutting is a very low rate of propagation which is about 1:6 to 1:8 [15] and 1:7 to 1:10[16,17]. In addition to low rate of propagation on an open field, it favors pathogens to keep on accumulating generation after generation which reduces the yield and quality of sugarcane [14,18]. For instance Ratoon stunting disease is a common disease in sugarcane and conventionally it is treated with hot water that could be ineffective or could damage the set [19]. Hence, availability of quality planting material of newly released varieties is a major constraint in their adoption and commercialization within a short period of time. The time spent for conventional multiplication is considered as a serious economic problem, mainly in view of the higher yields that would be obtained by planting the new variety earlier on a large commercial scale, therefore efficient propagation systems are mandatory for mass multiplication.

Tissue culture of sugarcane has got a considerable research attention because of its economic importance as a cash crop. Plant multiplication or regeneration via tissue culture is a viable alternative for improving the quality and quantity of sugarcane [20]. Plant tissue culture (Micro propagation) holds immense potential for mass multiplication, subsequent rejuvenation and quality production of sugarcane [21]. By *in vitro* propagation, it is possible to produce some 260,000 shoots in four months [22] and $2x10^8$ plantlets within 4-5 weeks [14] from single shoot tip of sugarcane. It is demonstrated that micro propagated system exhibited a potential *in vitro* production of 75600 shoots from a single shoot apex explant in a period of about 5.5 months [23]. It is reported that around 2500 seedlings could be generated from one bud in a 12 week period on MS medium supplemented with 1.5 mgL^{-1} of BAP and GA_3[24]. Rapid micro propagation is also achieved [25] by producing 78408 plantlets in three months on MS media supplemented with BAP (0.2 mgL^{-1}) and Kinetin (0.1 mgL^{-1}) and he conclude that by using tissue culture it would be possible to commercialize a new variety within 1-2 years. In Ethiopia, there is no sugar breeding facility and new varieties have be imported by sett and propagated for commercialization by cutting so far. Hence, this experiment was launched to optimize a protocol for *in vitro* mass propagation of newly introduced genotypes to supplement the conventional vegetative propagation.

Materials and Methods

The experiment was conducted at Plant Tissue Culture Laboratory of Jimma University College of Agriculture and Veterinary Medicine (JUCAVM), Ethiopia. Two sugarcane genotypes (C86-12 and C86-56) were used for the study. They were imported from Cuba in 2006 and passed through agronomic performance evaluation. They were among the selected ones to be commercialized. The sets of these genotypes were prepared and treated with hot water. The setts were taken to JUCAVM green house and planted. After two to three months of growing, shoot tip explants were taken from the sugarcane plants. The explants were prepared according to [14] procedures. The surrounding leaf sheaths of sugarcane tops were carefully removed one by one until the inner white sheaths were exposed. The explants were sized to about 10 cm length by cutting off at the two ends, locating the growing point somewhere near to top. They were washed under running tap water and liquid detergents. They were socked in fungicide solution (0.3% kocid) for 30 minutes under laminar flow cabinet containing three drops of tween-20. After the kocid was properly washed off from the explants, they were rinsed three times with distilled water and disinfected with 70% ethanol for one minute. The ethanol was poured off and the explants were rinsed again with sterile distilled water. Disinfection of explants was done with 0.1% of $HgCl_2$ for 10 minutes [26] followed by 3-4 washing with sterile distilled water. The required amounts of all stock solutions of MS [27] media, 30 gL^{-1} [28] sucrose and combinations of different concentrations of BAP and Kin were mixed in a beaker and the pH was adjusted to be 5.8. This was followed by addition of 0.8% agar for solidifying the media. Then, it was heated to melt the agar and 30 ml media was dispensed in to culture jars. Finally, it was autoclaved at temperature of 121°C for 20 minutes with 15 psi of pressure.

Initiated explants were cultured under laminar flow hood aseptically and transferred to the growth room at which growth conditions were adjusted to be 16 hours of photoperiod with 25 $\mu molm^{-2}s^{-1}$ photosynthetic photon flux intensity and 26 ± 2°C of temperature. The experiment was laid down in factorial treatment combination in complete randomized design with two factor factorial treatment combination arrangements. Each of treatment was replicated three times. Data on number of shoots per explant, number of leaves per shoot and shoot length were collected after 30 days of culturing. Finally data were subjected to two-way analysis of variance (ANOVA) using SAS statistical software version 9.2 (SAS Inc., 2008) and treatments' means were separated by using REGWQ (Ryan-Einot-Gabreil-Welsch Multiple range test) mean separation method.

Results and Discussions

Analysis of variance revealed that the interaction among BAP and Kin combinations and genotypes was highly significant (p=0.001) on number of shoots per explant, number of leaves per shoot and shoot length. MS medium without PGRs did not result in shoot multiplication on both genotypes (Table 1). However, increasing the concentration of kinetin from 0.0 mgL^{-1} to 1 mgL^{-1} without BAP increased the mean number of shoots per explant from 0.0 ± 0.0 to 10.2 ± 0.445 and 0.0 ± 0.0 to 13.6 ± 0.548 for C86-12 and C86-56 respectively. Similarly, increasing the concentration of BAP from 0.0 mgL^{-1} to 2.0 mgL^{-1} for C86-12 and 0.0 mgL^{-1} to 1.0 mgL^{-1} for C86-56 without Kin showed a significant increase in the mean number of shoots per explant from 0.0 ± 0.0 to 17.8 ± 0.447 and 0.00 ± 0.00 to 14.0 ± 0.707 respectively. This showed that addition of exogenous PGRs is a must to have shoot multiplication. Moreover, the increasing trend in shoot number per explant is due to the fact that cytokinin (BAP and Kin) stimulate protein synthesis and participate in cell cycle control in a cell division [29]. If cytokines are used for shoot culture media, they can overcome apical dominance and release lateral buds from dormancy and enhance shoot multiplication [29].

From the two genotypes, C86-12 gave higher mean number of shoots per explant (33.8 ± 0.837) with 13.04 ± 0.089 mean number of leaves per shoot and mean shoot length of 8.4 ± 0.008 cm on MS medium supplemented with 1.5 mgL^{-1} of BAP and 0.5 mgL^{-1} of Kin (Table 1 and Figure 1A). With the same medium composition, C86-56 gave only 17.4 ± 0.548 mean number of shoots per explant with 8.4 ± 0.548 mean number of leaves per shoot and 3.22 ± 0.567 cm mean shoot length. In this genotype, the highest mean number of shoots per explant (25.6 ± 0.548) was obtained with mean number of leaves per shoot of 9.8 ± 0.447 and mean shoot length of 8.65 ± 0.724 cm on MS medium supplemented with 1.5 mgL^{-1} BAP and 1.0 mgL^{-1} Kin(Table 1 Figure 1B). However, the same medium in C86-12 resulted in 20.0 ± 0.707 mean number of shoots per explant; 6.3 ± 0.447 mean number of leaves per shoot and 4.27 ± 0.013 cm mean shoot length. For C86-12, as the concentration of kinetin increased from 0.0 mgL^{-1} to 0.5 mgL^{-1}

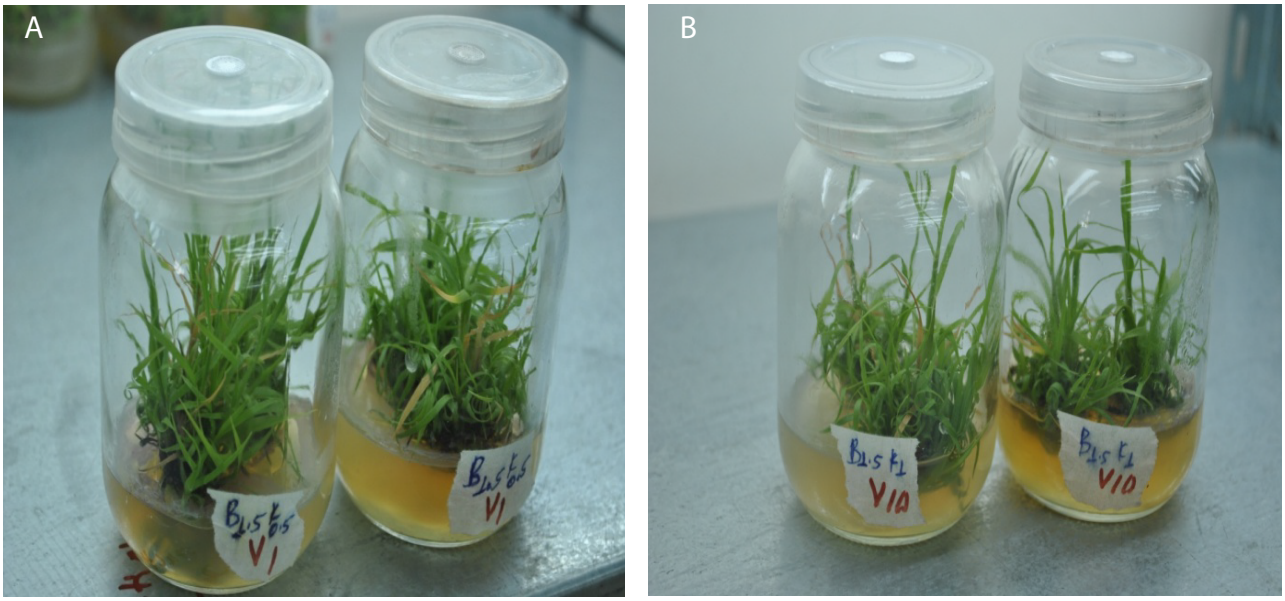

Figure 1: Shoot multiplication showing best results of
A: C86-12 genotype on MS medium containing 1.5 mgL^{-1} BAP and 0.5 mgL^{-1} Kin
B: C86-56 genotype on MS medium containing 1.5 mgL^{-1} BAP and 1 mgL^{-1} Kin.

PGRs(mgl^{-1})		C86-12			C86-56		
BAP	Kin	Number of shoots per explant ± SD	Number of leaves per shoot ± SD	Shoot length(cm) ± SD	Number of shoot per explant ± SD	Number of leaves per shoot ± SD	Shoot length(cm) ± SD
0	0	0.0^{s} ± 0.000	0.0^{p} ± 0.00	0.0^{v} ± 0.00	0.00^{s} ± 0.00	0.00^{p} ± 0.00	0.00^{v} ± 0.00
0	0.1	2.2^{rs} ± 0.447	3.9^{o} ± 0.224	3.52^{m-p} ± 0..013	3.00^{qr} ± 0.00	3.98^{o} ± 0.044	3.04^{p-t} ± 0.089
0	0.5	8.2^{p} ± 0.0.447	5.1^{mn} ± 0.224	7.04^{bc} ± 0.089	5.2^{q} ± 0.447	5.08^{mn} ± 0.179	4.04^{h-n} ± 0.094
0	1	10.2^{n-p} ± 0.445	6.06^{i-m} ± 0.134	4.72^{e-g} ± 0.013	13.6^{h-m} ± 0.548	4.7^{no} ± 0.975	5.74^{d} ± 0.004
1	0	12.8^{i-n} ± 0.433	7.8^{f-h} ± 0.477	5.47^{de} ± 0.241	14.0^{h-l} ± 0.707	9.28^{b-e} ± 0.438	4.44^{g-j} ± 0.458
1	0.1	16.2^{f-h} ± 0.447	7.1^{g-i} ± 0.894	5.8^{d} ± 0.811	9.1^{op} ± 0.224	8.24^{e-g} ± 0.537	4.57^{f-i} ± 0.297
1	0.5	15.1^{g-i} ± 0.548	10.4^{b} ± 0.548	6.76^{c} ± 1.327	20.2^{cd} ± 0.834	9.42^{b-e} ± 0.83	5.19^{f-i} ± 0.495
1	1	15.0^{g-i} ± 1.225	9.4^{b-e} ± 0.548	5.22^{d-f} ± 0.367	17.8^{d-f} ± 1.095	8.9^{c-f} ± 0.549	3.77^{j-p} ± 0.223
1.5	0	15.9^{f-h} ± 0.224	8.0^{f-h} ± 0.000	3.82^{i-o} ± 0.008	12.2^{j-n} ± 0.447	8.0^{f-h} ± 0.000	3.28^{n-r} ± 0.008
1.5	0.1	16.9^{fg} ± 0.224	9.0^{c-f} ± 0.000	4.04^{h-n} ± 0.089	14.0^{h-l} ± 0.00	8.8^{d-f} ± 0.447	3.59^{l-p} ± 0.004
1.5	0.5	33.8^{a} ± 0.837	13.04^{a} ± 0.089	8.4^{a} ± 0.008	17.4^{e-g} ± 0.548	8.4^{ef} ± 0.548	3.22^{o-s} ± 0.567
1.5	1	20.0^{cd} ± 0.707	6.3^{kl} ± 0.447	4.27^{g-m} ± 0.013	25.6^{b} ± 0.548	9.8^{b-d} ± 0.447	8.65^{a} ± 0.724
2	0	17.8^{d-f} ± 0.447	9.2^{b-e} ± 0.433	4.48^{f-j} ± 0.171	12.8^{i-n} ± 0.447	7.00^{h-j} ± 0.707	3.29^{n-q} ± 0.350
2	0.1	16.0^{f-h} ± 0.00	7.88^{f-h} ± 0.521	3.45^{n-p} ± 0.172	12.8^{i-n} ± 1.095	6.4^{kl} ± 0.548	3.91^{i-o} ± 0.004
2	0.5	12.8^{i-n} ± 0.447	6.62^{lj} ± 0.567	4.31^{g-l} ± 0.050	17.0^{fg} ± 2.121	6.4^{kl} ± 0.548	4.41^{g-j} ± 0.004
2	1	10.2^{n-p} ± 0.447	9.78^{b-d} ± 0.491	4.84^{e-g} ± 0.014	11.8^{k-n} ± 0.837	9.00^{c-f} ± 0.00	4.89^{e-g} ± 0.007
2.5	0	11.0^{m-o} ± 0.00	6.16^{i-m} ± 0.447	2.57^{q-t} ± 0.108	12.8^{i-n} ± 0.447	6.6^{lj} ± 0.548	2.52^{st} ± 0.005
2.5	0.1	11.2^{m-o} ± 0.447	6.84^{h-j} ± 1.314	2.41^{tu} ± 0.101	14.2^{g-j} ± 1.923	6.4^{kl} ± 0.548	2.67^{q-t} ± 0.004
2.5	0.5	10.2^{n-p} ± 0.837	5.26^{k-n} ± 0.581	2.42^{tu} ± 0.121	12.4^{i-n} ± 3.647	5.8^{j-n} ± 0.447	2.73^{q-t} ± 0.039
2.5	1	17.2^{e-g} ± 0.447	7.16^{g-i} ± 0.851	4.96^{e-g} ± 0.604	14.8^{h-k} ± 3.271	6.6^{e-l} ± 0.547	4.38^{g-k} ± 0.000
3	0	20.6^{c} ± 0.548	10.02^{bc} ± 0.447	7.46^{b} ± 0.380	13.2^{i-m} ± 2.588	5.2^{l-m} ± 0.447	3.96^{i-o} ± 0.054
3	0.1	12.8^{i-n} ± 0.00	6.34^{kl} ± 0421	3.64^{k-p} ± 0.215	19.6^{c-e} ± 3.286	6.66^{lj} ± 0.615	3.74^{j-p} ± 0.004
3	0.5	12.0 ± 0.447	6.46^{j-k} ± 0.639	2.55^{r-t} ± 0.152	15.08^{g-i} ± 0.179	7.1^{g-i} ± 0.224	2.71^{q-t} ± 0.004
3	1	11.4^{i-o} ± 0.548	6.6^{lj} ± 0.616	2.38^{tu} ± 0.225	11.4^{i-o} ± 0.548	6.00^{j-m} ± 0.00	1.73^{u} ± 0.02
CV	(%)	8.45	7.25	7.89	8.45	7.25	7.89

Table 1: Effects of 6-benzylaminopurine and kinetin on shoot multiplication

PGRs=Plant growth regulators. Values for number of shoots per explant, number of leaves per shoot and shoot length given as mean ± SD. Numbers with in the same column with different letter(s) are significantly different from each other according to REGWQ at $p \leq 0.05$.

keeping BAP at 1.5 mgL^{-1}, the mean numbers of shoots per explant, mean number leaves per shoot and mean shoot length showed a significant increase from 15.9 ± 0.224 to 33.8 ± 0.837, 8.0 ± 0.000 to 13.04 ± 0.089 and 3.82 ± 0.008 to 8.4 ± 0.008 respectively. However, for C86-56 only mean number of shoots per explant showed a significant increment from 12.2 ± 0.447 to 17.4 ± 0.548.

The best result obtained in C86-12 is in agreement with the result reported by [30]. They reported that optimum multiplication from HSF-240 genotype exhibited 16.5 cm mean shoot length, 11 number of shoots per explant and 32 leaves per explant on medium supplemented with 1.5 mgL^{-1} BAP, 0.5 mgL^{-1} Kin and 30 gL^{-1} sucrose after 30 days of culturing. Though they found higher number of leaves per shoot and shoot length, the present study is better in terms of mean shoot number per explant. This difference could be due to genotypic difference. The best results in both genotypes of the present study contradict with results reported in [31-33]. Best results were obtained from CO678 genotype on MS medium supplemented with 2 mgL^{-1} BAP+0.5 mgL^{-1} Kin with 9.1 ± 0.1 mean number of shoots, 6.83 ± 0.12 mean shoot length and 5.67 ± 0.04 leaves per shoot. He also obtained 7.87 ± 1.06 mean number of shoots, 5.44 ± 0.19 mean number of leaves and 6.33 ± 0.21 mean shoot length on MS medium supplemented with 2 mgL^{-1} BAP+0.25 mgL^{-1} kin+30 gL^{-1} sucrose from Co449 genotype but from both genotypes he reported much less number of shoot per explant than the result of this study[31]. Tilahun M (2011) [31] reported more number of shoots per explant (34 ± 1.54) than the current result but with less number of leaves per shoot (12 ± 0.17) and shoot length (6.95 ± 0.01 cm) on MS medium supplemented with 1 mgL^{-1} BAP+0.5 mgL^{-1} Kin+30 gL^{-1} sucrose for B41-227 genotype. Comparable mean shoot number per explant (29.7 ± 1.0069) from BL-4 genotype on MS medium supplemented with 0.25 mgL^{-1} BAP and Kin each was reported [8]. These differences happened because it is an established fact that different genotypes may give different results on MS medium supplemented with different concentrations of plant growth regulator and combinations. Sharma M [33] found 20 ± 0.15; 24 ± 0.22 mean number of shoots per explant and 7.0 ± 0.27; 7.4 ± 0.06 mean shoot length for CoJ 83 and CoS 8436 genotypes respectively after 21 days of culturing on MS medium supplemented with 1.0 mgL^{-1} BAP+1.5 mgL^{-1} Kin+30 gL^{-1} sucrose . The difference is not only due to genotypic variation but also due to the number of days taken for culturing.

1. Therefore, the best results obtained on MS medium supplemented with1.5 mgL^{-1} BAP and 0.5 mgL^{-1} kin for B85-12 and 1.5 mgL^{-1} BAP and 1 mgL^{-1} kinetin for C86-56 showed genotypic difference in relation to concentrations of BAP and Kinetin combinations to be used for optimal shoot multiplication. It is because of the fact that different genotypes possess specific receptor proteins and differed in concentration for plant growth regulators [29]

Conclusion

The present results showed that MS medium fortified with 1.5 mgL^{-1} BAP and 0.5 mgL^{-1} of Kin for B86-12; and 1.5 mgL^{-1} of BAP and 1.0 mgL^{-1} of Kin for C86-56 were found to be the optimum media for shoot multiplication. Hence, by using these media combinations (protocol), these genotypes can be commercialized within a short period of time and supplement the conventional propagation which improves both the quality and quantity of the planting materials.

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References

1. Ather A, Khan S, Rehman A, Nazir M (2009) Optimization of the Protocols for Callus induction, Regeneration and Acclimatization of sugarcane cv. Thatta-10. Pak. J. Bot. 41: 815-820.
2. Naturland EV (2000) Organic farming in the tropics and Sub tropics. Kleinhaderner, Germany 10-15.
3. Sengar K, Garg SK (2011) Role of tissue culture technique in high sugarcane production. A review Life Sciences 1008-1017.
4. Snyman SJ, Meyer GM, Koch AC, Banasiak M, Watt MP (2011) Applications of in vitro culture systems for commercial sugarcane production and improvement. In Vitro Cell. Biol. Plant. 47: 234-249
5. Duri M (1969) Private Foreign Investment in Ethiopia. J. Eth Studies 7: 53-73.
6. Pandey RN, Rastogi J, Sharma ML, Singh K (2011) Technologies for Cost Reduction in Sugarcane Micro propagation. African Journal of Biotechnology 10: 7814-7819.
7. Gallo-Meagher M, English RG, Abouzid A (2000) Thidiazuron Stimulates Shoot Regeneration of Sugarcane Embryogenic Callus. In Vitro Cell Dev Biol-Plant 36: 37-40.
8. Ali A, Naz Sh, Siddiqui FA, Iqbal J (2008) An Efficient Protocol for Large Scale Production of Sugarcane through Micropropagation. Pak. J. Bot. 40: 139-149.
9. Anonymous (2010) F.O, licht's International Sugar and Sweetener Report.
10. Malik KB (1990) Proposal for Approval of BF-162. Sugarcane Research Institute, Faisalabad 7-10.
11. Manickavasagam M, Ganpati A, Anbazhagan VR, Sudhakar B, Selvaraj N, et.al. (2004) Agrobacterium mediated genetic transformation and development herbicide resistant sugarcane (Saccharum species hybrids) using auxiliary buds. Plant Cell Rep. 23: 134-43.
12. Cox M, Hogarth M, Smith G (2000) Cane breeding and improvement. Manual of cane growing, Bureau of sugar Experimental Stations, Indooroopilly, Australia.
13. Birader S, Biradar DP, Patil VC, Patil SS, Kambar NS (2009) In vitro plant regeneration using shoot tip culture in commercial cultivar of sugarcane. Karnataka J. Agric. Sci. 22: 21-24.
14. Cheema KL, Hussain M (2004) Micropropagation of Sugarcane through Apical Bud and Axillary Bud. Int J Agric Biol 6: 257-259.
15. Jalaja NC, Neelamathi D, Sreenivasan, TV (2008) Micropropagation for quality seed Production in Sugarcane in Asia and the Pacific. Sugarcane pub 13-60.
16. Dash M, Mishra PK, Mohapatra D (2011) Mass propagation via shoot tip culture and detection of genetic variability of *Saccharium Officinarum* clones using biochemical markers. Asian Journal of Biotechnology 10:1996-0700.
17. Gosal SS, Thind KS, Dhaliwal HS (1998) Micropropagation of sugarcane an efficient protocol for commercial plant production. Crop Impro. 25: 167-171.
18. Nand L, Singh HN (1994) Rapid clonal multiplication of sugarcane through tissue culture. Plant Tissue Cult 4:1-7.
19. Hoy JW, Bischoff KP, Milligan SB, Gravois KA (2003) Effect of tissue culture explant source on sugarcane yield components. Euphytica 129: 237-240.
20. Baksha R, Alam R, Karim MZ, Paul SK, Hossain MA, et.al. (2002) In vitro Shoot Tip Culture of Sugar-cane (*Saccharium officinarum*) Variety Isd. Biotechnology 1: 67-72.
21. Heinz DJ, Mee GWP (1969) Plant differentiation from callus tissue of *Saccharium* species. Crop Sci. 9: 346-348.
22. Hendre RR, Iyer RS, Kotwal M (1983) Rapid multiplication of sugarcane by tissue culture. Sugarcane 1: 58.
23. Lal J, Pande HP, Awasthi SK (1996) A general Micropropagation protocol for sugarcane varieties. New Bot. 23: 13-19.
24. Chattha MA, Imran MI, Abida A, Muhammad I, Akhtar A (2001) Micropropagation of sugarcane (Saccharum sp.). Pakistan Sugar J. 16: 2-6.
25. Lee TSG, (1987) Micropropagation of sugarcane (*Saccharium* sp.) Plant Cell, Tissue and Organ culture 10: 47-55.
26. Bisht SS, Routray AK, Mishra R (2011) Rapid in vitro propagation techniques for sugarcane variety 018. IJPBS 2: 0975-6299.

27. Murashige T, Skoog F (1962) A Revised Medium for Rapid Growth and Bioassay with Tobacco Cultures. Physiol. Plant. 15: 473-479.

28. Khan SA, Rashid A, Chaudhary, MF, Chaudhry Z, Afroz A (2008) Rapid Micropropagation of three elite Sugarcane (*Saccharium officinarum L.*) varieties by shoot tip Culture. AJB 7: 2174-2180.

29. George EF, Machakova I, Zazimalova E (2008) Plant propagation by tissue culture. 3rd edition 175-205.

30. Khan SA, Rashid H, Chaudhary MF, Chaudhry Z, Fatima Z et.al. (2009) Effect of Cytokinin on Shoot Multiplication in Three Elite Sugarcane Varieties. Pak. J. Bot. 41: 1651-1658.

31. Tilahun M (2011) Protocol Optimization for In Vitro Mass Propagation of Two Sugarcane (Saccharum officinarum L.) Clones Grown in Ethiopia. An MSc Thesis Presented to School of Graduates Studies of Jimma University College of Agriculture and Veterinary Medicine.

32. Belay T, Mulugeta, B and Derbew, B (2014) Effects of 6-Benzyl amino purine and Kinetin on In Vitro Shoot Multiplication of Sugarcane (Saccharum officinarum L.) Varieties. Adv Crop Sci. Tech. 2: 129

33. Sharma M (2005) In vitro regeneration studies of sugarcane. An MSc Thesis, Patiala, India 24-32.

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