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Response of Sugarcane (*Saccharum officinarum L.*) Varieties to BAP and IAA on *In vitro* Shoot Multiplication

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Abstract

In spite of its diverse limitations, the conventional propagation method is exclusively used for multiplication of sugarcane planting materials in the Ethiopian Sugar Estates since the establishment of the sugar industry in 1954. The present study was carried out to optimize *in vitro* shoot multiplication protocol for two selected sugarcane varieties (B41-227 and N14) widely grown in Ethiopian sugar estates to complement with the conventional propagation method. In the study, initiated aseptic shoot tip cultures of the two sugarcane varieties were treated with four concentrations (1.5, 2, 2.5 and 3 mgL^{-1}) of 6- benzylaminopurine (BAP) and Indole-3-acetic acid (IAA) (0.25, 0.5, 0.75 and 1 mgL^{-1}) while Plant growth regulator free medium was used as free check (control). The experiment was set up in a completely randomized design (CRD) with three factor factorial treatment combinations arrangements. Data were collected on number of shoots per explant, average shoot length and number of leaves per shoot after 30 days. Data were subjected to three way analysis of variance. The study verified that medium fortified with 1.5 mgL^{-1} 6-benzylaminopurine (BAP) and 0.5 mgL^{-1} indole-3-acetic acid (IAA) for B41-227 and 2 mgL^{-1} 6-benzylaminopurine (BAP) and 0.25 mgL^{-1} indole-3-acetic acid (IAA) for N14 resulted in optimum multiplication responses. On these media, B41-227 produced 15.5 ± 2.90 shoots per explant with 5.93 ± 0.57 cm average shoot length and 6.4 ± 1.49 leaves per shoot while N14 gave 11 ± 00 shoots per explant with 6.32 ± 0.23 cm average shoot length and 5.8 ± 0.06 leaves. Thus, the optimized protocol can be used for rapid *in vitro* mass multiplication of the sugarcane varieties and hence minimize the limitations sugarcane planting materials.

Keywords: Conventional propagation; *In vitro* shoot multiplication; Sugarcane; BAP; IAA

Introduction

Sugarcane is the most important cash crop widely grown in tropical and sub tropical regions of the world and is the major source of sugar [1-3]; accounting for 70% of the world's total sugar production [3-7]. Properties such as an efficient photosynthesis and efficient biomass production make this crop an excellent target for industrial processing and valuable alternative and prime candidate for ethanol and other byproducts production [8,9]. The Ethiopia sugar industry utilizes only sugarcane and has great contributions to the socio-economy of the country. It produces sugar for the household and industrial consumption, provides job opportunity for the nationals, produces ethanol and serves as a source of energy and co-products used for miscellaneous purposes [10].

However, the current sugar production of the Ethiopian Sugar Industry covers only 60% of the annual demand for domestic consumption while the deficient is imported from abroad. In order to make the country self sufficient in sugar and export the surplus sugar and produce ethanol and other by-products, the Federal government of Ethiopia is working to establish sugarcane plantation on 325,000 ha in addition to the vast expansion project of the previously established farms with erection of high crashing capacity 10 new sugar mills. However, availability of adequate amount of quality disease free planting materials of sugarcane within a short time is the major limiting factor. In addition, the yield of the existing few and old commercial cane varieties is declining sharply and some productive varieties were obsolete due to lack of alternative technologies for disease cleansing and rejuvenation. Moreover, commercialization of improved introduced and adapted sugarcane varieties took several years through the conventional propagation method, challenging the realization of the intended plan through conventional propagation method.

In conventional propagation method where stem cuttings with two to three nodes used as a planting material have various limitations. A bud produces 4 to 5 shoots [4] and the rate of propagation is 1:10 in a year [11,12]. In contrast, if estimated conservatively, micropropagation can produce 10,000 identical plants from a single bud in about 3 to 4 months [13] and the rate of propagation is 1:22 to 1:25 in 8 to 10 months [14]. Propagation from stem cuttings facilitates spread of pathogens with accumulation of disease over vegetative cycles leading to reduction in yield and quality [11,12]. Unlike the conventional propagation method, micropropagation using shoot tip or apical meristem culture have been widely used to produce virus-free plants [15-18] with rapid multiplication of new variety [19-21] and for rejuvenation and mass production of true to type and uniform planting materials from old diseased sugarcane plants [22,23]. Moreover, tissue culture raised sugarcane plants were reported to give superior cane and sugar yield as compared to their donors from conventional seed source under similar climatic conditions and agronomic management practices [24-28]. Thus, it is imperative to optimize *in vitro* propagation protocol to minimize the challenges of conventional propagation method and utilize the merits of micropropagation technology. In addition, there is no information on tissue culture study of sugarcane varieties grown in Ethiopian Sugar estates. Therefore, this study was carried out with the objective to evaluate the response of the two sugarcane varieties to different levels BAP and IAA on *in vitro* shoot multiplication.

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Materials and Methods

The study was conducted at plant tissue culture laboratory of Jimma University College of Agriculture and Veterinary Medicine, Ethiopia. Two sugarcane varieties, B41-227 and N14, were used in this study. These varieties were collected from Metahara and Wonji-Shoa sugar estate seedcane nurseries of the Ethiopian Sugar Corporation. The stock plants were planted after hot water treatment (52°C for 2 hours) and grown under greenhouse conditions for two to three months. Then, preparation of explants was carried out according to the standard procedure of [12,29] with some modifications. Shoot tops were cut from actively growing sugarcane plants grown in the greenhouse. The leaves were removed and the shoot tops were taken to the laboratory. In the laboratory, surrounding leaf sheaths were removed carefully 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. The shoot tops were washed under running tap water for one minute with soap solution and treated with 0.3% Kocide (fungicide solution) for one and half an hour under laminar air flow cabinet. After decanting Kocide solution, shoot tops were washed three times with sterile distilled water and further immersed in 70% ethanol for one minute and rinsed three times each for five minute with sterile distilled water. Finally, the explants treated with 10% (v/v) Sodium hypochlorite solution (4% w/v 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. The surface sterilized explants were excised and sized to 1 cm long and 0.5 cm diameter and cultured on initiation media. The initiated aseptic cultures were used to set up the multiplication experiment. Murashige and Skoog (1962), (MS) media, in full strength was used with different concentrations and combinations of 6-benzylaminopurine (BAP) and Indole-3-acetic acid (IAA). The medium contained 30 g/l sucrose as a carbon source and the pH was adjusted to 5.8 before gelling with 8 g/l agar and autoclaved at 121°C and 15 psi for 20 minutes. Molten medium of 40 ml was dispensed per each culture jar.

The experiment was carried out at growth room temperature range of 23-27°C under 16-hours light and eight hours dark photoperiod regimes maintained under fluorescent light having 2500 µmolm-2S-1 light intensity and 75-80% relative humidity. The experiment was laid out in a factorial treatment combination in a completely randomized design with a three factor factorial treatment combinations arrangement. Data were subjected to three way analysis of variance (ANOVA) using SAS statistical software *version 9.2* (SAS Institute Inc., 2008) and Treatments' means were separated using the procedure of REGWQ (Ryan-Einot-Gabriel-Welsch Multiple range test).

Results and Discussion

Analysis of variance (ANOVA) revealed that there was a very high significant interaction among the sugarcane varieties, BAP and IAA (vareity *BAP *IAA=p<0.0001) on the number of shoots per explant, average shoot length and number of leaves per shoot in both sugarcane varieties. On MS media lacking plant growth regulators BAP and IAA (control), no multiple shoot formation occurred in any of the varieties tested. B41-227 gave the highest (15.5 ± 2.90) number of shoots per explant on MS medium supplemented with 1.5 mgL^{-1} BAP and 0.5 mgL^{-1} IAA (Table 1 and Figure 1) while N14 produced only 3.22 ± 1.67 shoots per explant on this medium composition. N14 produced maximum of 11 ± 0.00 shoots per explant on MS medium containing 2 mgL^{-1} BAP and 0.25 mgL^{-1} IAA (Table 1 and Figure 2) while the same medium composition resulted in only 5.83 ± 0.93 shoots per explant in B41-227. B41-227 also gave the highest average shoot length (5.93 ± 0.23 cm) with the largest number of 6.4 ± 1.49 leaves per shoot on MS medium containing 1.5 mgL^{-1} BAP + 0.5 mgL^{-1} IAA where only 2.92 ± 1.27 cm average shoot length with 3.5 ± 1.11 leaves per shoot observed in N14. In B41-227, maintaining BAP at 1.5 mgL^{-1} while increasing IAA levels from 0.25 to 0.5 mgL^{-1}, showed a marked increase in the number of shoots per explant from 4 ± 0.69 to 15.5 ± 2.90, average shoot length from 4.25 ± 1.02 to 5.93 ± 0.57 cm and number of leaves per explant from 3.52 ± 0.19 to 6.4 ± 1.49. However, further increase in the levels of IAA to 0.75 mgL^{-1} significantly reduced the number of shoot per explant, average shoot length and number of leaves per shoot to 7.0 ± 1.37, 5.17 ± 0.53 cm and 4.33 ± 0.11, respectively. The rate

PGRs (mg/l)		B41-227			N14		
BAP	IAA	Number of shoots per explant	Shoot length (cm)	Number of leaves per shoot	Number of shoots Per explant	Shoot length (cm)	Number of leaves per shoot
1.5	0.25	$4.00^{n} \pm 0.69$	$4.25^{gh} \pm 1.02$	$3.52^{l} \pm 0.19$	$2.80^{t} \pm 0.88$	$2.35^{no} \pm 0.54$	$3.33^{m} \pm 0.42$
	0.5	$15.50^{a} \pm 2.90$	$5.93^{b} \pm 0.57$	$6.40^{a} \pm 1.49$	$3.22^{qr} \pm 1.67$	$2.92^{l} \pm 1.27$	$3.50^{l} \pm 1.11$
	0.75	$7.00^{c} \pm 1.37$	$5.17^{d} \pm 0.53$	$4.33^{h} \pm 0.11$	$4.50^{m} \pm 0.77$	$4.37^{g} \pm 1.19$	$4.45^{g} \pm 0.78$
	1	$6.21^{d} \pm 0.21$	$4.74^{e} \pm 0.27$	$4.50^{fg} \pm 0.54$	$5.80^{f} \pm 1.11$	$5.23^{d} \pm 1.18$	$3.35^{m} \pm 0.68$
2	0.25	$5.83^{f} \pm 0.93$	$4.55^{f} \pm 0.19$	$3.81^{j} \pm 0.05$	$11.00^{b} \pm 0.00$	$6.30^{a} \pm 0.23$	$5.80^{b} \pm 0.06$
	0.5	$10.90^{b} \pm 0.82$	$5.26^{d} \pm 1.35$	$5.20^{d} \pm 0.08$	$5.41^{hi} \pm 1.91$	$3.78^{i} \pm 1.01$	$5.22^{d} \pm 0.40$
	0.75	$6.02^{e} \pm 1.26$	$5.85^{b} \pm 0.00$	$4.50^{fg} \pm 0.89$	$4.64^{l} \pm 0.71$	$3.42^{j} \pm 0.44$	$4.00^{j} \pm 0.90$
	1	$5.52^{gh} \pm 0.77$	$5.52^{c} \pm 1.30$	$4.33^{h} \pm 0.41$	$3.22^{rs} \pm 0.92$	$2.88^{l} \pm 0.83$	$3.54^{l} \pm 0.78$
2.5	0.25	$6.00^{e} \pm 0.38$	$4.17^{h} \pm 1.46$	$4.51^{f} \pm 0.91$	$3.33^{q} \pm 1.22$	$4.15^{h} \pm 0.64$	$2.90^{o} \pm 0.62$
	0.5	$5.24^{j} \pm 0.65$	$3.82^{i} \pm 0.04$	$3.33^{m} \pm 0.60$	$3.80^{o} \pm 0.69$	$3.23^{k} \pm 0.00$	$4.82^{e} \pm 0.00$
	0.75	$5.20^{j} \pm 0.82$	$3.52^{j} \pm 0.31$	$5.51^{c} \pm 1.27$	$4.10^{n} \pm 0.54$	$2.25^{o} \pm 0.47$	$3.00^{n} \pm 0.81$
	1	$4.81^{k} \pm 0.21$	$4.38^{g} \pm 0.67$	$4.34^{h} \pm 1.11$	$4.43^{m} \pm 0.00$	$5.21^{d} \pm 0.66$	$2.42^{r} \pm 0.66$
3	0.25	$4.53^{lm} \pm 0.55$	$3.19^{k} \pm 1.02$	$3.50^{l} \pm 0.00$	$3.52^{p} \pm 0.00$	$4.53^{f} \pm 0.31$	$3.84^{j} \pm 0.11$
	0.5	$4.50^{m} \pm 0.67$	$2.93^{l} \pm 0.79$	$3.50^{l} \pm 0.15$	$3.33^{q} \pm 0.48$	$2.65^{m} \pm 0.00$	$3.60^{k} \pm 0.00$
	0.75	$3.17^{rs} \pm 1.20$	$3.00^{l} \pm 0.15$	$3.33^{m} \pm 0.97$	$2.80^{t} \pm 0.01$	$2.11^{p} \pm 0.19$	$2.82^{p} \pm 0.92$
	1	$3.05^{s} \pm 0.55$	$2.44^{n} \pm 0.44$	$2.92^{o} \pm 0.24$	$2.52^{u} \pm 0.51$	$2.46^{n} \pm 0.00$	$2.50^{q} \pm 0.71$
CV (%)		8.33	5.27	7.91	8.33	5.27	7.91

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

Table 1: Response of B41-227 and N14 to BAP and IAA on In vitro shoot multiplication

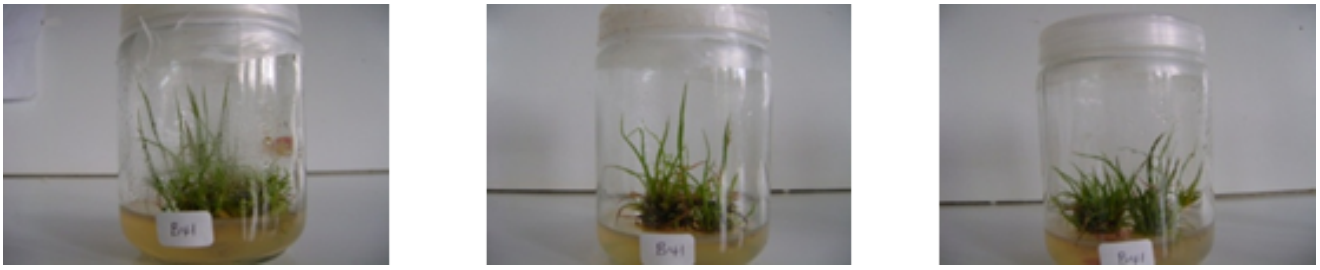

Figure 1: *In vitro* shoot multiplication of B41-227 at 1.5 mg/l BAP and 0.5 mg/l IAA

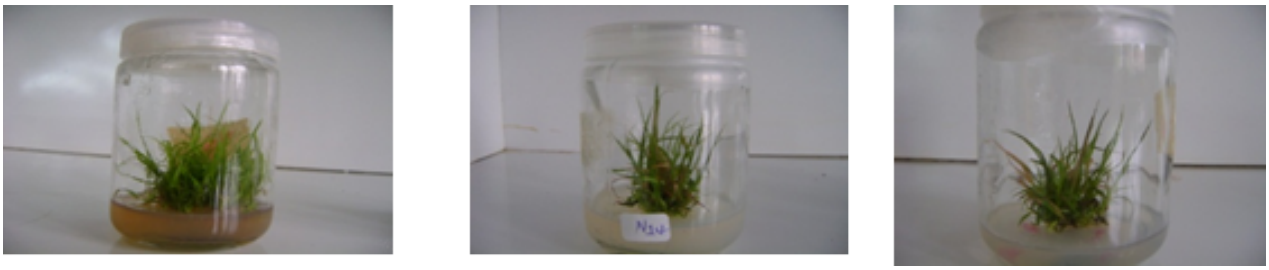

Figure 2: *In vitro* shoot multiplication of N14 at 2 mg/l BAP and 0.25 mg/l IAA

of sugarcane propagule multiplication depends upon auxin-cytokinin balance of culture medium [30]. A low concentration of auxin is often beneficial in conjunction with higher levels of cytokinin during shoot multiplication and exogenous auxin does not promote auxiliary shoot proliferation; however, their presence in culture medium may improve the culture growth [31]. Although cytokinins are known to stimulate cell division, but does not induce DNA synthesis. Nevertheless, the presence of auxin promotes DNA synthesis. Hence, the presence of auxin together with Cytokinin stimulates cell division and control morphogenesis thereby influences shoot multiplication. The present results in B41-227 are in line with the findings of [32] in terms of the number of shoots per explant. He found maximum of 16.5 shoots per explant on MS medium supplemented with 3 mgL^{-1} BAP with 1 mgL^{-1} IAA in sugarcane, but in contrast with the present result in N14. Murashige and Skoog (MS) medium supplemented with BAP and IAA for shoot induction in sugarcane showed the formation of profuse shoots on the MS medium containing 1 mgL^{-1} BAP along with 0.5 mgL^{-1} IAA [33,34].

Conclusion

Based on the current result, it is possible to deduce that, we have developed a rapid *in vitro* shoot multiplication protocol for the two sugarcane varieties which can use to complement the conventional propagation method. Murashige and Skoog (1962) (MS) media fortified with 1.5 mgL^{-1} benzylaminopurine (BAP) and 0.5 mgL^{-1} indole-3-acetic acid (IAA) for B41-227 and 2 mgL^{-1}benzylaminopurine (BAP) and 0.25 mgL^{-1} indole-3-acetic acid (IAA) for N14 can be used as a suitable medium combinations for rapid shoot multiplication *in vitro*.

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References

1. Bahera KK, Sahoo S (2009) Rapid *in vitro* micropropagation of sugarcane (Saccharum officinarum L.cv-Nayana) through callus culture. Nature and science 7: 1545-0740.
2. Sajid GM, S Pervaiz (2008) Bioreactor mediated growth, culture ventilation, stationary and shake culture effect on *in vitro* growth of sugarcane. Pak J Bot 40: 1949-1956.
3. Suprasana P (2010) Biotechnological Interventions in sugarcane improvement: Strategies, methods and progress. Nuclear Agriculture and Biotechnology Division, Technology Development Article 316.
4. Khan S A, Rashid A, Chaudhary MF, Chaudhary Z, Afroz A (2008) Rapid Micropropagation of three elite Sugarcane (SaccharumofficinarumL.) varieties by shoottip culture. African Journal of Biotechnology 7: 2174-2180.
5. Anonymous (2005) The biology of Saccharum spp. (sugarcane). Department of health and Aging, office of the gene technology.
6. Ali A, Naz S, Iqbal J (2007) Effect of different explants and media compositions for efficient somatic embryogenesis in sugarcane (Saccharum officinarum L.). Pak J Bot 39: 1961-1977.
7. FAO (2013) Food and Agriculture Organization of the united States of America. World sugarcane production statistics.
8. Ather A, Khan S, Rehman A, Nazir M (2009) Optimization of the protocol for callus induction, regeneration and acclimatization of sugarcane cv.THATTA-10. Pak J Bot 41: 815-820.
9. Gallo-Meagher, R G. English, A Abouzid (2000)Thidiazuron stimulates shoot regeneration of sugarcane embryonic callus. *In vitro* cellular and developmental biology plant 36: 37-40.
10. Ambachew D, Firehun Y (2010) Cane sugar productivity potential in Ethiopia. Second Biannual Conference ESI 4-6
11. Biradar S, Biradar BP, Patil VC, Kambar NS (2009) *In vitro* plant regeneration using shoot tip culture in commercial cultivars of sugarcane. Karnataka Journal of Agric Scie 22: 21-24.
12. Jalaja NC, Neelamathi D, Sreenivasan TV (2008)Micropropagation for quality seed Production in sugarcane in Asia and the Pacific. Sugarcane pub 13-60.
13. Lee TSG (1987) Micropropagation of sugarcane (Saccharum spp). Plant cell Tissue Org Cul 10: 47-55.
14. Tawar PN (2004) Sugarcane Seed multiplication and Economics. National Training course on sugarcane micropropagation. VSI, Pune, India.
15. Hendre RR, Iyer RS, Kotwal M (1983) Rapid multiplication of sugarcane by tissue culture. Sugarcane 1: 58.

16. Parmessur Y, Aljanabi A, Saumtally S, Dookun-Saumtally A (2002) Sugarcane yellow leaf virus and sugarcane yellows phytoplasma: Elimination by tissue culture. Plant pathology 51: 561-566.

17. Fitch MMM, Leherer AT, Komor E, Moore PH (2001) Elimination of sugarcane yellow leaf virus from infected sugarcane plants by meristem tip culture visualized by tissue blot immunoassay.

18. Lal J, Pande HP, SK Awasthi (1996) A general micro propagation protocol for sugarcane varieties. New Bot 23: 13-19.

19. Anita PJ, Sehrawat RK, AR, Punia A (2000) Efficient and cost effective micropropagation of two early maturing varieties of sugarcane (Saccharum Spp.). India sugar 50: 611-618.

20. Sandhu SK, Gossal SS, Thind KS, Uppal SK, Sharma B, et al. (2009) Field performance of micrpropagated plants and potential of seed cane for stock yield and quality in sugarcane. Sugar tech research article11: 34-38.

21. Singh N, Kumar A, Garg GK (2006) Genotype influence of phytohormone combination and sub culturing on Micropropagation of sugarcane varieties. Indian Journal of biotechnology 5: 99-106.

22. Heinz DJ, Mee GW (1969) Plant differentiation from callus tissue ofSaccharum species. Crop sci 9: 346-348.

23. Lakshmanan (2012) Sugarcane tissue culture. Sugarcane for the future. Information sheet IS13034.

24. Anonymous(2002) Micropropagation: Tissue culture techniques in sugarcane. Indian Institute of sugarcane Research, Directorate of sugarcane Development1-2.

25. Comstock JC, Miller J (2004) Yield comparison: Disease free tissue cultures versus bud propagated planted sugarcane plants and healthy versus yellow leaf virus infected plants. USDA-ARS, Sugarcane field station, canal point, Florida 33438.

26. Geetha S, Padmanabhan D (2001) Effect of hormones on direct somatic embryogenesis in sugarcane. Sugar Tech 3: 120-121.

27. Nand L, Ram K (1997) Yield comparison in sugarcane crop raised from conventional and mericlone derived seedcane.Ind Sugar 47: 617-621.

28. Ramanand, Lal M, Singh S (2005) Comparative performance of micropropagated and conventionally raised crops of sugarcane. Sugar tech 7: 93-95.

29. Singh R (2003) Tissue culture studies of sugarcane. M.Sc. Thesis submitted to Thapar Institute of Engineering and Technology: 18-22.

30. Soodi N, Gupta PK, Srivastava RK, Gosal SS(2006) Comparative studies on field performance of micrpropagated and conventionally propagated sugarcane plants. Plant tissue cult & Biotech16: 25-29.

31. Kumari R, Verma DK(2001) Development of micropropagation protocol for sugarcane (Saccharum officinarum L)-A review. Agric Reve 22: 87-94.

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

33. Dhumale ED, Engoe GL, Durge DV (1994) *In vitro* regeneration of sugarcane by tissue culture. Ann Pl Phys 8: 192-194.

34. Larkin PJ (1982) Sugarcane tissue and protoplast culture. Plant Cell Tissue Org. Cul 1: 149-164.

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