Molecular Biology: Open Access

Tolera and Terefe, Mol Biol 2017, 6:2
DOI: 10.4172/2168-9547.1000186

Research Article Open Access

Interaction Effects of 6-Benzylaminopurine (BAP) and Indole-3-Butyric Acid (IBA) on *Ex Vitro* Propagation of Sugarcane

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Received date: January 23, 2017; **Accepted date:** January 30, 2017; **Published date:** February 06, 2017

Abstract

Ex vitro propagation of micropropagated sugarcane plantlets of three selected sugarcane genotypes was carried out with the objective of evaluating their propagation responses to the interaction effects of BAP and IBA. Accordingly, six levels of IBA (0.0, 0.1, 0.2, 0.3, 0.4 and 0.5 mg L^{-1}) and eight levels of BAP (0.0, 0.75, 1.0, 1.25, 1.5, 1.75, 2.0 and 2.25 mg L^{-1}) with three sugarcane genotypes, each replicated three times were tested. The treatments were arranged in a factorial completely randomized design. Data on the number of tillers per shoot, average shoot length (cm) and number of active leaves per shoot were collected twice every 30th day after 30 days of treatment application. Analysis of variance revealed that the interaction effects of BAP, IBA and the sugarcane genotypes was very highly significant (P<0.0001) on the number of tillers per shoot, average shoot length and number of active leaves per shoot. The optimum number of tillers per shoot (5.67), average shoot length (40.77 cm) and number of active leaves per shoot (6.97) were obtained at 0.1 mg/l IBA+0.75 mg/l BAP in C132-81. In C86-56, the optimum number of tillers per shoot (4.50) was obtained at 0.1 mg/l IBA+1.5 mg/l BAP while the maximum average shoot length (48.33 cm) and maximum number of active leaves per shoot (7.67) were obtained at 0.2 mg/l IBA+0.75 mg/l BAP. In SP70-1284, the optimum number of tillers per shoot (6.43) was obtained at 0.3 mg/l+1 mg/l BAP with 16.05 cm average shoot length and 4.87 active leaves per shoot. Thus, it can be deduced that production of an average of 5.5 plantlets per shoot within a month can be possible.

Keywords: *Ex vitro* propagation; IBA; BAP; Sugarcane genotypes; C132-81, C86-56; SP70-1284

Introduction

Sugarcane is a perennial grass which produces seed under suitable conditions, but for commercial production, it is propagated from stalk cuttings. Propagation through stalk cuttings is the traditional method of sugarcane propagation in which stalk cuttings containing one or more buds, termed sets are used for commercial planting [1]. The Ethiopian Sugar Industry used this traditional method of propagation alone for the last 50 years till 2012. However, under the new plan of the Industry to expand the existing farms and establishment of vast sugarcane plantation projects with many high crushing capacity sugar factories, the tradition propagation method alone was seen to have various draw backs. Among these, availability of enough amount of quality disease free planting material within short time, transport of bulky unclean seed cane from existing farms to the remote project farms, the low rate of propagation(usually 1:10), lack of methods for fast commercialization of improved and adapted varieties, obsolation of productive commercial varieties due to disease, lack of alternative techniques for rejuvenation and disease cleansing of the old contaminated sugarcane varieties were the major limitations identified as challenges to the vast expansion and new development plans [2,3]. With a view to minimize the challenges, microproagation technology was adopted and implemented to supplement the tradition method of sugarcane propagation in all sugar estates and projects. Microproagation Technology is a technique through which group of genetically identical plants all derived from a selected individual multiply vegetatively and rapidly by aseptic culture of meristematic regions under defined nutritional and controlled environmental conditions *in vitro* [4]. Nowadays, unlike the traditional propagation method, it is the only practical means of achieving rapid and large scale production of disease free quality planting materials in sugarcane [5-7] and alternative approach for fast multiplication of a genotype in its original form. It is very effective in entire disease cleansing, rejuvenation and subsequent mass propagation of well adapted and promising varieties facing gradual deterioration in yield, quality and vigor due to accumulation of pathogens during prolonged vegetative cultivation and hence sustains the productive potential of sugarcane crops for a longer period [8,9]. Furthermore, micropropagated sugarcane plants were reported to give superior in cane and sugar yield as compared to their donors under similar agronomic management systems [10-14]. However, the Ethiopian Sugar

Corporation is procuring the micropropagated sugarcane plantlets from different organizations where the cost of procurement is high (about US$ 0.205 per plantlet). The erratic supply, long distance transport of the delicate plantlets followed by reduced survival, the increasing demand of micropropagated plants with the subsequent procurement cost increment were found to be the major limitations for the profitability and sustainability of the micropropagation technology. Therefore, this experiment was carried out with the objective to evaluate the effects of 6-benzylaminopurine (BAP) in vivo propagation of tissue culture raised sugarcane plantlets of three sugarcane genotypes (C132-81; C86-56 and SP70-1284) with a view to complement microproagation technology and to ensure continuous supply, cut down the cost of plantlets procurement and propagation of

sufficient amount of quality planting materials at the farm gate nursery within short period of time.

Materials and Methods

The study was carried out at Metahara sugarcane plantation, located at Eastern part of the country, at about 200 km away from Addis Ababa, Ethiopia. Metahara Sugar estate is situated at 80 53' N latitude and 390 52' E longitudes at an altitude of 950 m above sea with a semi-arid climatic condition. The experimental materials were *in vitro* propagated sugarcane genotypes of C132-81, C86-56 and SP70-1284. The primary acclimatized plantlets of these sugarcane varieties were delivered with intact coco-peat from Mekelle Technology Institute Tissue Culture Laboratory and directly planted in white polyethylene bag (8cm diameter with 10 cm height) filled with mixture of Luvisol, sand and compost in the ratio of 8:2:1. The experimental design was Completely Randomized Design with factorial treatment combination arrangements. Three sugarcane genotypes (C132-81, C86-56 and SP70-1284) with six levels of IBA (0.0, 0.1, 0.2, 0.3, 0.4 and 0.5 mg L^{-1}) and eight levels of BAP (0.0, 0.75, 1.0, 1.25, 1.5, 1.75, 2.0 and 2.25 mg L^{-1}) resulting in 144 treatment combination arrangements each replicated three times. Each plot contains 30 pots (one plantlet a pot) and data on the number of tillers per shoot, shoot length and number of active leaves per shoot were collected from ten randomly selected plantlets at 30th and 60th days of treatment application. Then the average data were subjected to analysis of variance using SAS software version 9.2 while separation of significant means' was done using REGWQ (Ryan-Einot-Gabriel- Welsch) Multiple Range Test.

Results and Discussion

Analysis of variance revealed that the interaction of Indole-3-butyric acid (IBA), 6-benzylaminopurine (BAP) and sugarcane genotype have a very highly significant (BAP*IBA*Genotype=p<0.001) effect on all the response variables tested: numbers of leaves per shoot, average shoot length and number of leaves per shoot in all the sugarcane genotypes tested: C132-81, C86-56 and SP70-1284 (Table 1).

Source of Variation	DF	Mean Square		
		Number of Tillers/Shoot	Average Shoot length(cm)	Number of leaves/shoot
IBA	4	1.05*	43.08*	0.54**
BAP	6	2.82***	709.77***	1.89***
IBA*BAP	24	1.11***	71.49***	0.43***
Gen	2	45.34***	1110.21***	24.17***
IBA*Gen	8	0.83*	16.36ns	0.12ns
BAP*Gen	12	2.26***	142.78***	0.98***
IBA*BAP*Gen	48	2.64***	30.31***	1.36***

Remark: DF: Degree of Freedom; *** P ≤ 0.0001; ** P>0.0001 and P<0.001; * P>0.001 and P<0.05; ns: P>0.05; IBA: Indole-3-Butyric Acid; BAP: 6-Benzyleaminopurine; Gen: Genotype

Table 1: Summary for ANOVA on the interaction effects of BAP, IBA and sugarcane genotypes.

Comparison of the sugarcane genotypes revealed that all the three sugarcane genotypes showed marked variation in all the responses tested: number of tillers per shoot, average shoot length and number of leaves per shoot (Table 2). Regardless of the other treatment, comparison of the sugarcane genotypes shows us SP70-1284 produced the highest number of tillers per shoot C86-56 gave the highest average shoot length (37.28 cm) and maximum number of leaves (6.36) per shoot (Table 2). In sugarcane genotype C132-81, the lowest number of tillers per shoot (1.63) was found on 0 mg/l IBS+0 mg/l BAP (control treatment) with 27.27 cm average shoot length and 4.00 leaves per shoot. However, increasing the concentration of IBA from 0 mg/l to 0.1 mg/l and BAP from 0 mg/l to 0.75 mg/l, increased the number of tillers per shoot from 1.63 to 5.67, average shoot length from 27.72 cm to 40.77 cm and number of leaves from 4.00 to 6.97. The maximum number of tillers per shoot (5.67), highest average shoot length (40.77 cm) and number of leaves per shoot (6.97) was obtained at 0.1mg/l IBA and 0.75 mg/l BAP in sugarcane genotype C132-81 (Table 3). Holding the concentration of BAP at 0.75 mg/l and increasing the concentration of IBA beyond 0.1 mg/l up to 0.5 mg/l showed a declining trend in number of tillers per shoot, average shoot length (cm) and number of leaves per shoot.

Genotype	Number of tillers/ shoot	Average shoot length(cm)	Number of leaves/ shoot
C86-56	3.02c	37.28a	6.36a
C132-81	3.20b	32.94b	6.14b
SP70-1284	4.45a	30.13c	5.19c

Table 2: Comparison of sugarcane genotypes to the interaction effects of IBA, BAP and sugarcane genotypes.

Similarly, at 1 mg/l BAP, increasing the concentration of IBA from 0.1 up to 0.3 mg/l showed an increasing trend in the number of tillers per shoot and number of leaves per shoot while further increase revealed a decreasing effect in both response variables (Table 3). For the response variable shoot length, increasing the concentrations of IBA from 0.1 up to 0.5 at 1 mg/l BAP showed a decreasing effect. Generally, for in vivo proliferation of sugarcane genotype C132-81, 0.1 mg/l IBA with 0.75 mg/l BAP give the optimum growth response for number of tillers per shoot, average shoot length (cm) and number of leaves per shoot. Further increase in either of the growth regulators or

both have no any biological and economic advantage (Table 3). In sugarcane genotype C86-56, the lowest number of tillers per shoot (1.50) and average shoot length (22.67 cm) was found on the control treatment (0 mg/l IBA and BAP) while the lowest number of leaves per shoot (4.10) was obtained at 0.5 mg/l IBA and 2.25 mg/l BAP (Table 3). In C86-56, increase in the number of tillers per shoot from 1.50 to 2.67; average shoot length from 22.67 cm to 34.00 cm and number of leaves per shoot from 4.70 to 7.00 was obtained as a result of 0.1 mg/l IBA+0.75 mg/l BAP (Table 3). At 0.75 mg/l BAP, increasing the concentration of IBA from 0.1 mg/l to 0.2 mg/l, increased the number of tillers per shoot, average shoot length and number of leaves per shoot from 2.67 to 33, 34 cm to 42 cm and 7.00 to 7.67, respectively. However, further increase in the concentration of IBA at 0.75 mg/l BAP has no positive effect neither of the response variables (number of tillers per shoot, average shoot length and number of leaves per shoot) tested. For this genotype (C86-56), the maximum number of tillers per shoot (4.50) was obtained at 0.1 mg/l IBA+1.5 mg/l BAP while the highest average shoot length (48.33 cm) and maximum number of leaves per shoot (7.67) was obtained at 0.5 mg/l IBA+1.5 mg/l BAP and 0.2 mg/l IBA+0.75 mg/l BAP, respectively. In sugarcane genotype SP70-1284, the lowest number of tillers per shoots (2.50) was obtained on the control treatment (0 mg/l IBA+0 mg/l BAP) while the maximum number of tillers per shoot (6.43) was obtained at 0.3 mg/l IBA along with 1.0 mg/l BAP with 16.05 cm average shoot length and 4.87 leaves per shoot. At 1.0 mg/l BAP, further increase in the concentration of BAP from 0.3 mg/l to 0.4 mg/l decreased the number of tillers from 6.43 to 5.57 (Table 3). The rate of sugarcane propagules multiplication depends upon auxin - cytokinine balance of culture medium [11]. A low concentration of auxin is often beneficial in conjunction with higher levels of cytokinine during shoot multiplication and exogenous auxin does not promote auxiliary shoot proliferation; however, their presence in culture medium may improve the culture growth. Although cytokinines 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 Cytokinine stimulates cell division and control morphogenesis thereby influences shoot multiplication [2,3].

PGRs (mg/l)			C132-81			C86-56			SP70-1284	
IBA	BAP	Number of tillers/ shoot	Average shoot length(cm)	Number of leaves/ shoot	Number of tillers/shoot	Average shoot length(cm)	Number of leaves/ shoot	Number of tillers/ shoot	Average shoot length(cm)	Number of leaves/shoot
0	0	1.63^{z}	27.27^{w}	4.00^{e}	1.50^{z}	21.67^{y}	4.70^{fg}	2.50^{w}	26.33^{x}	6.00^{c}
0.1	0.75	5.67^{c}	40.77^{w}	6.97^{ab}	2.67^{u}	34.00^{n}	7.00^{ab}	5.63^{c}	29.23^{t}	5.13^{f}
0.2	0.75	3.20^{op}	36.87^{k}	6.80^{b}	3.33^{o}	42.00^{f}	7.67^{a}	4.53^{g}	31.13^{s}	5.40^{e}
0.3	0.75	2.80^{j}	30.50^{s}	5.90^{c}	3.33^{o}	37.00^{k}	6.33^{bc}	3.67^{k}	25.83^{x}	5.60^{d}
0.4	0.75	2.70^{t}	29.67^{t}	5.17^{f}	3.33^{o}	31.33^{s}	6.67^{ab}	4.10^{i}	26.57^{x}	5.73^{cd}
0.5	0.75	2.63^{v}	29.03^{u}	4.93^{fg}	2.67^{u}	29.33^{t}	6.33^{bc}	3.73^{k}	29.60^{t}	5.13^{f}
0.1	1	2.60^{jk}	36.80^{k}	6.03^{c}	2.43^{x}	44.67^{c}	6.00^{c}	3.23^{o}	41.30^{g}	5.07^{f}
0.2	1	2.60^{jk}	34.40^{n}	6.47^{b}	2.90^{s}	47.67^{b}	6.03^{c}	3.70^{k}	32.50^{r}	4.83^{fg}
0.3	1	3.73^{k}	29.13^{u}	6.23^{bc}	3.00^{r}	33.67^{n}	6.67^{ab}	6.43^{a}	16.05^{z}	4.87^{fg}
0.4	1	3.53^{l}	27.07^{w}	6.13^{bc}	3.33^{r}	32.00^{p}	6.33^{bc}	5.57^{d}	37.80^{j}	5.23^{f}
0.5	1	3.20^{op}	27.00^{w}	6.00^{c}	3.33^{r}	30.67^{s}	6.00^{c}	4.90^{ef}	26.70^{x}	5.53^{cd}
0.1	1.25	2.43^{x}	27.33^{w}	5.87^{c}	2.67^{u}	28.33	5.67^{cd}	4.07^{i}	25.40^{y}	5.13^{f}
0.2	1.25	2.40^{x}	27.50^{v}	5.90^{c}	3.33^{o}	29.67^{t}	6.33^{bc}	4.00^{i}	29.40^{t}	5.17^{f}
0.3	1.25	2.93^{s}	33.17^{o}	6.00^{c}	3.67^{k}	37.00^{k}	6.00^{c}	4.13^{i}	41.30^{g}	5.07^{f}
0.4	1.25	3.07^{q}	35.73^{j}	6.63^{b}	3.67^{k}	37.33^{j}	6.83^{ab}	4.13^{i}	34.17^{n}	4.97^{f}
0.5	1.25	3.43^{n}	36.27^{k}	6.40^{b}	3.67^{k}	37.33^{j}	7.00^{ab}	5.33^{e}	24.67^{xy}	5.00^{fg}
0.1	1.5	2.87^{s}	34.60^{m}	6.03^{c}	4.50^{g}	47.67^{b}	8.00^{a}	5.73^{b}	24.60^{xy}	5.27^{f}
0.2	1.5	2.93^{r}	27.77^{w}	$6.10b^{c}$	3.33^{r}	25.67^{xy}	6.33^{bc}	4.03^{i}	25.97^{y}	5.57^{vd}
0.3	1.5	2.57^{v}	28.30^{u}	$6.27b^{c}$	3.37^{r}	28.67^{u}	5.67^{cd}	4.33^{h}	26.73^{x}	5.67^{d}
0.4	1.5	2.60^{v}	33.53^{n}	5.80^{c}	3.60^{k}	38.33^{j}	6.67^{ab}	4.37^{h}	37.47^{j}	4.93^{fg}
0.5	1.5	2.77^{t}	35.27^{n}	5.53^{d}	3.67^{k}	48.33^{a}	6.00^{c}	4.33^{h}	37.67^{j}	5.00^{f}

0.1	1.75	4.00^{i}	37.40^{j}	6.60^{b}	3.47^{m}	43.33^{e}	5.97^{c}	4.37^{h}	31.33^{s}	5.00^{f}
0.2	1.75	3.00^{r}	32.67^{p}	6.13^{bc}	3.33^{o}	41.33^{g}	7.33^{ab}	5.67^{d}	26.33^{s}	5.00^{f}
0.3	1.75	3.00^{r}	32.90^{o}	6.33^{bc}	2.67^{u}	33.00^{n}	6.00^{c}	5.00^{e}	26.40^{s}	5.27^{f}
0.4	1.75	2.70^{u}	28.53^{u}	6.27^{bc}	2.63^{u}	32.07^{r}	6.33^{c}	3.83^{j}	24.00^{xy}	5.50^{cd}
0.5	1.75	2.20^{y}	27.27^{w}	5.80^{c}	2.33^{u}	32.00^{r}	6.00^{c}	3.57^{l}	22.60^{y}	5.10^{fg}
0.1	2	2.53^{w}	32.20^{q}	5.70^{cd}	3.33^{o}	40.33^{h}	6.67^{ab}	4.53^{g}	36.90^{k}	4.87^{fg}
0.2	2	2.87^{t}	38.20^{j}	5.93^{c}	3.33^{o}	47.67^{b}	6.67^{ab}	4.00^{i}	39.80^{i}	5.37^{f}
0.3	2	3.17^{op}	32.53^{q}	6.27^{bc}	3.03^{o}	42.00^{f}	6.73^{ab}	3.60^{k}	31.63^{s}	4.93^{fg}
0.4	2	3.20^{op}	26.73^{w}	5.97c^{d}	3.33^{o}	37.00^{k}	7.00^{ab}	3.57^{kl}	22.93^{y}	4.90^{fg}
0.5	2	2.83^{t}	37.73^{j}	6.50^{b}	2.67^{u}	25.67^{xy}	6.67^{ab}	3.10^{r}	22.60^{y}	5.60^{cd}
0.1	2.25	2.23^{y}	33.17^{o}	5.50^{d}	2.67^{u}	30.67^{s}	6.00^{c}	4.03^{i}	26.30^{x}	5.30^{d}
0.2	2.25	2.10^{y}	34.33^{n}	5.60^{c}	2.67^{u}	38.67^{j}	6.00^{c}	4.47^{g}	28.70^{v}	4.87^{fg}
0.3	2.25	2.47^{w}	31.67^{s}	5.87^{c}	3.33^{o}	44.00^{c}	5.67^{cd}	4.60^{f}	39.83^{i}	4.97^{fg}
0.4	2.25	2.97^{r}	30.77^{s}	4.03^{ab}	3.27^{op}	47.33^{b}	4.33^{ab}	4.17^{i}	39.67^{i}	5.03^{fg}
0.5	2.25	2.40^{w}	30.67^{s}	3.60^{cd}	2.67^{k}	41.00^{g}	4.10^{ab}	4.00^{i}	26.27^{x}	5.73^{cd}
CV		7.6	12.7	6.6	7.6	12.7	6.6	7.6	12.7	6.6
Mean ± SE		0.36	2.45	0.22	0.36	2.45	0.22	0.36	2.45	0.22
Values (Mean ± SE) in the table indicates superscripts with the same letter are not significantly different										

Table 3: Growth response of micropropagated sugarcane plants to the interaction effects of IBA and BAP.

Conclusion

The traditional method of sugarcane planting material propagation has many limitations while microproagation technology is efficient to solve all the limitations except it is costly to produce or procure the micropropagated plantlets. Hence, *ex vitro* propagation technology (IVPT) was developed to complement tissue culture technology, but reproducibility of the IVPT protocols is dependent on the genotype, environmental weather conditions, soil mixture used, plant growth regulators and interaction of the factors used. Therefore, the aim of this study was to optimize in vivo propagation protocol for three recently released sugar genotypes: C132-81, C86-56 and SP70-1284. The result proved that *ex vitro* propagation of the sugarcane genotypes tested is highly dependent on the interaction effects of IBA, BAP and the sugarcane genotypes. Treatment combination containing 1 mg/l BAP and 1.5 mg/l gave optimum propagation responses. Sugarcane genotype C132-81 4 tillers per shoot with 30.67 cm average shoot length and 6 active leaves per shoot while C86-56 produced 3.53 and 4.67 tillers per shoot, 29.33 cm average shoot length and 7.0 and 6.33 active leaves per shoot per month, respectively. Thus, the current finding will help minimize the current challenges of sugarcane production by rapidly availing adequate amount of quality planting material of sugarcane while reducing the cost of plantlets procurement and hence the cost of sugar production.

In sugarcane genotype C132-81, the optimum number of tillers per shoot (5.67), highest average shoot length (40.77 cm) and number of active leaves per shoot (6.97) was obtained at 0.1 mg/l IBA and 0.75 mg/l BAP while in C86 - 156), the optimum number of tillers per shoot (4.50) was obtained at 0.1 mg/l IBA+1.5 mg/l BAP while the optimum average shoot length (48.33 cm) and maximum number of leaves per shoot (7.67) was obtained at 0.2 mg/l IBA+0.75 mg/l BAP. However, in SP70-1284, the optimum number of tillers per shoot (6.43) was obtained at 0.3 mg/l IBA+1.0 mg/l BAP with 16.05 cm average shoot length and 4.87 leaves per shoot. Thus, from this result, it can be deduced that this result can be used to produce sufficient quantity (5 plantlets per shoot within a month) of quality planting materials and therefore can guarantee sustainable supply of planting materials with the lowest possible cost.

References

1. Ridge R (2013) Fertilizing for high yield and quality in sugarcane. IPI Bulletin No.21. Australia.
2. Tolera B (2015) *In vivo* proliferation of in vitro propagated sugarcane (*Saccharum officinarum* L.) genotypes at Tana- Beles sugar development project, North-Western Ethiopia. Cell Dev Biol 4: 161.
3. Tolera B, Diro M, Belew D (2014) Response of sugarcane (*Sacharum officinarum* L.) varieties to BAP and IAA on *in vitro* shoot multiplication. Advances in Crop Science Technology 2: 126
4. Prakash J BS, Savangikar VA, Savangikar C (2002) Low cost options for tissue culture technology in developing countries Proceedings of a Technical Meeting organized by the Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture and held in Vienna, pp: 26–30.
5. Lal N, Krishna R (1994) Sugarcane and its problems: Tissue Culture for Pure and Disease Free Seed Production in Sugarcane. Indian Sugar 44: 847-848.

6. Lorenzo JC, Ojeda E, Espinosa A, Borroto C (2001) Field performance of temporary immersion bioreactor derived sugarcane plants. *In Vitro* Cell Dev Biol-Plant 37: 803-806.
7. Krisnamurthi M, Tlaskal J (1994) Fiji disease resistant *Saccharum officinarum* L. var. Pindar sub clone from tissue culture. Proc ISSCT 13: 130-137.
8. Sreenivasan TV, Sreenivasan J (1992) Microproagation of sugarcane varieties for increasing cane yield. SISSTA Sugar J 18: 61-64.
9. (2002) Microproagation: A tissue culture technique in sugarcane. Indian Institute of Sugarcane Research.
10. Ibrahim M, Tolera B, Aman J, Negi T (2016) Evaluation of tissue culture raised sugarcane planting materials against their donor conventional seed sources as initial sources of seed cane at Tendaho sugar development project, North-Eastern Ethiopia. Journal of Horticulture 3: 168.
11. Soodi N, Gupta PK, Srivastava RK, Gosal SS (2006) Comparative studies on field performance of micropropagated and conventionally propagated sugarcane plants. Plant Tissue Cult Biotech 16: 25-29.
12. Comstock JC, Miller JD (2004) Yield comparison: Disease free tissue cultures versus bud propagated planted sugarcane plants and healthy versus yellow leaf virus infected plants. Journal of American Society and Sugarcane Technologies 24: 31-40.
13. Cox M, Hogarth M, Smith G (2000) Cane breeding and improvement. In: Manual of cane growing, Hogarth M and P Allsopp P (ed.). Bureau of Sugar Experimental Stations, Indooroopilly, Australia pp: 91-108.
14. Geetha S, Padmanabhan D (2001) Effect of hormones on direct somatic embryogenesis in sugarcane. Sugar Tech 3: 120-121.