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EFFECT OF CYTOKININS ON SHOOT MULTIPLICATION IN THREE ELITE SUGARCANE VARIETIES

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Abstract

Studies were carried out for rapid micropropagation of three elite sugarcane varieties *i.e*., HSF-240, CP-77-400 and CPF-237. The explants were surface sterilized with 50% clorox for 30 minutes. The cultures were initiated by inoculating them on MS (Murashige & Skoog, 1962) medium containing 1.0 mg/l Kin along with 0.1 mg/l GA_3. Multiplication of the cultures was obtained by using BAP and Kin in various combinations and concentrations in MS medium. The optimum multiplication for variety HSF-240 was obtained at 1.5 mg/l BAP, 0.5 mg/l Kin with 16.5 cm shoot length, 11 number of tillers and 32 number of leaves per plant. Similarly optimum multiplication for variety CP-77-400 was obtained at 1.0 mg/l BAP and 0.5 mg/l Kin, with a maximum of 8.5 cm shoot length, 7 number of tillers and 24 number of leaves. Best multiplication rate for variety CPF-237 was observed at 1.0 mg/l BAP and 0.1 mg/l Kin with a maximum of 12 cm shoot length, 6 number of tillers and 18 leaves per plant. Rooting of the plantlets was obtained on half strength MS medium containing 6% sucrose and various concentrations of IBA.

Introduction

Sugarcane (*Saccharum officinarum* L.) is the fourth major crop of Pakistan and has paramount importance among the cash crops. Commercially, sugarcane is propagated from stem cuttings with each cutting or set having two or three buds. After the establishment of variety, major bottleneck in spreading of the variety is slow propagation rate through conventional method, which takes years (Cheema & Hussain, 2004). Modern commercial sugarcane varieties are obtained through breeding and a multi-stage selection scheme over a period of 10-15 years. Tissue culture techniques have been widely used for large-scale micropropagation and can effectively reduces the time period between selection and commercial release of new sugarcane varieties (Lorenzo *et al*., 2001; Taylor, 1997). Micropropagation is currently the only realistic means of achieving rapid, large-scale production of disease-free seed canes of newly developed varieties in order to speed up the breeding and commercialization process in sugarcane (Feldmann *et al*., 1994; Lal & Krishna, 1994). In contrast to conventional method where one bud produces 4-5 shoots, tissue culture, if estimated conservatively can produce around 10,000 identical plants from a single bud in about 3-4 months (Lee, 1987). Micropropagation can also be used for obtaining disease free plants. Many scientists have described methods for micropropagation (Hendre *et al*., 1983, Gosal *et al*., 1998, Jadhav *et al*., 2001). Gosal *et al*., (1998) reported rapid multiplication in liquid MS medium on BAP (0.5 mg/l) and Kin (0.5 mg/l) and rooting on NAA (0.5 mg/l) and sucrose 70%. In the present study we have developed protocol for fast and efficient micropropagation of three sugarcane varieties viz., HSF-240, CP-77-400 and CPF-237 by using BAP and Kinetin in various concentrations and combinations, which will help to cope with the increasing demand for sugarcane production on large scale and will meet the future challenges for sugar production.

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Materials and Methods

The plant materials were provided by the Sugar crops programme at Crop Sciences Institute and all the experimental work was carried out at Agriculture Biotechnology Programme (ABP), National Agriculture Research Centre (NARC), Islamabad. Three sugarcane varieties HSF-240, CP-77-400 and CPF-237 were used in this study. The explant materials were taken from six months old sugarcane plants. Size of the shoot tip taken was 4-6 mm. For surface sterilization of explants 50% clorox (commercial bleach containing 5.25% v/v Sodium hypochlorite) for 30 minutes was used but before clorox treatment the explants were first thoroughly washed with tap water for 30 minutes, then put in 70% ethanol for 45 minutes and finally in antioxidants solution for 30 minutes. After that the explants were washed with autoclaved distilled water three times each for 10 minutes. Solid MS (Murashige & Skoog`s medium, 1962) supplemented with 0.1 mg/l GA_3 and 1.0 mg/l Kin for initiation of cultures, various concentrations and combinations of BAP and Kin in liquid MS medium for the multiplication of cultures and with 0.5 mg/l-1.5 mg/l of IBA with 6% sucrose for the rooting of cultures. Data in case of shoot initiation were recorded 20 days after culturing, for shoot multiplication 30 days and 20 days for rooting after culturing. Statistical package MSTAT-C (1991) was used for the statistical analysis of data.

Results and Discussion

a. Initiation of cultures: Optimum shoot growth was recorded at 1.0 mg/l Kin in combination with 0.1 mg/l GA_3 for all the three varieties used in this study. The percentage growth for all the three varieties ranged from 70%-85% with 7.5cm-9cm shoot length and 4-7 number of leaves (Table 2b). Hendre *et al.,* (1983) obtained shoots of sugarcane cultivars on different concentrations of BAP and Kin. Maximum of 85% growth, 9cm shoot length and 7 leaves per plant were recorded for var. HSF-240. Similarly a total of 70% growth, 7.5cm shoot length and 4 number of leaves per plant were observed for var. CP-77-400, while a maximum of 80% growth, 8cm shoot length and 6 number of leaves were observed for var. CPF-237 (Table 2b). Razi-ud-Din *et al*., (2004) reported a maximum of 3.2 cm average shoot length on MS medium containing 1.5 mg/l GA_3 and 0.5 mg/l BAP.

b. Effects of different concentrations/combinations of BAP and Kin on multiplication: Multiplication of shoots was observed at different concentrations of BAP in combination with Kin. There were differences in the varietals response towards the different concentrations used however BAP (0.5-1.5 mg/l) with Kin (0.1-0.5 mg/l) were found best for multiple shoots formation. A maximum of 13 cm shoot length, 7 tillers and 26 leaves per plant were observed at 1.0 mg/l BAP in combination with 0.1 mg/l GA_3 in 30 days.

The average shoot length of shoot tips from varieties HSF-240, CP-77-400 and CPF-237 were highly significantly ($p<0.01$) different. The shoot length ranged from 5.2 cm for variety CP-77-400 to 10.5 cm for variety HSF-240. Baksha *et al*., (2002) also obtained a maximum of 5 ± 0.7 number of shoots with 4.0 ± 0.12 average shoot length in 10 days. The impact of different hormones combinations on average shoot length was also highly significantly ($p<0.01$) different. The percentage shoot length ranged from 1.2 cm to 10.17 cm. The control (T_5) showed the lowest value (1.2 cm) and T_3 showed the highest value of 10.17 cm (Table 1). Geetha *et al*., (2000) achieved multiplantlets of sugarcane on medium with BAP @ 1 mg/l. The interaction effect of varieties and hormones combinations was highly significant ($p<0.01$) on average shoot length. The shoot length for interaction between varieties and different

hormones combinations ranged from 1.0 cm for variety HSF-240 in control (T_5) to 16.5 cm again for variety HSF-240 in T_4 (Table 1). Mamun *et al*., (2004) used BA @ 1.5 mg/l for the production of higher percentage of shoot proliferation.

The impact of different varieties on number of tillers from shoot tips were non-significant ($p>0.05$). However the number of tillers ranged from 9.8 in variety CPF-237 to 11.6 in CP-77-400 (Table 1). The different hormones combinations showed highly significant ($p<0.01$) impact on average number of tillers. The number of tillers ranged from 0.16 to 6.16. The highest value 6.16 was observed for both T_3 and T_4 while the lowest one was for that of control (T_5, Table 1). Rapid shoot multiplication was achieved in liquid MS medium supplemented with cytokinins, Kin (0.5 mg/l) and BAP (0.5 mg/l) with 20 shoots per plant (Gosal *et al.,* 1998).

The interaction effect between varieties and different hormones combinations was non-significantly ($p>0.05$) different on tillers number. However the tillers number ranged from 0.0 to 11.0. The highest value of 11.0 was observed for that of variety HSF-240 with T_4 while the lowest value 0 was for that of both HSF-240 and CP-77-400 in control (T_5, Table 1). While Gallo-Meagher *et al*., (2000) reported TDZ for highest number of shoots production in sugarcane.

The average number of leaves for different varieties (HSF-240, CP-77-400, CPF-237) were highly significantly ($p<0.01$) different. The means for number of leaves ranged from 9.8 for variety CPF-237 to 17.5 for that of variety HSF-240 (Table 1). Chattha *et al.,* (2001) reported that multiple shoots formation was excellent at 1.5 mg/l BA in combination with 1.5 mg/l GA_3 and recorded a maximum of 4 tillers and 16 leaves per plant. The impact of different hormones combinations was also highly significant ($p<0.01$) against leaves number. However the leaves number ranged from 1.83 as the lowest value for control (T_5) to 18.83 as the highest value for T_3 (Table 1). Patel *et al.,* (2001) recorded highest multiplication on 1.5 mg/l Kin. The combinations T_2, T_3 and T_4 were non-significantly different while the combinations T_1 and T_5 were significantly different (Table 1). The interaction effects of varieties and different hormones combinations were significant ($p<0.05$) for number of leaves. The means for leaves number from shoot tips, for interaction effects of varieties and hormones combinations ranged from 1.5 to 32.5. The highest value (32 .5) was observed for HSF-240 at T_4 and the control (T_5) showed the lowest value (1.5) for variety CPF-237 (Table 1). While Wongkaew & Fletcher (2004) reported multiplication on 0.5 mg/l BAP along with 0.5 mg/l NAA and 15% coconut water.

c. Rooting of sugarcane varieties: Optimum roots induction was observed at 0.5 mg/l-1.5 mg/l IBA for the three varieties. Maximum roots (35) and root length (3.05 cm) was observed at 0.5 mg/l IBA for variety HSF-240 (Table 2a). In contrast Baksha *et al*., (2002) used 5 mg/l IBA and obtained 12 $\pm$ 0.2 average number of roots/shoot with 3 $\pm$ 0.1 cm average root length in a period of 10 days. For variety CP-77-400 a maximum of 41 roots were observed at 1.0 mg/l IBA with 2.05 cm root length at 0.5 mg/l IBA (Table 2a). Similarly var. CPF-237 best rooted at 1.5 mg/l IBA with 34 roots and 1.8 cm root length (Table 2a). While Karim *et al*., (2002) reported optimum root induction at 3 mg/l IBA with 11 roots per shoot along with 3.8 cm root length in 12 days.The rooted plantlets were transferred in flasks containing simple water. These flasks were covered with polythene bags for high humidity for 2-3 days. After that these were transferred to soil in growth room for hardening and then were transferred to green house to assess their potential for further hardening. The acclimatization potential was 70-80%.

Table 1. Effects of different combinations/concentrations of BAP + Kin on multiplication of three sugarcane cultivars HSF-240, CPF-237 and CP-77-400.

	Mean shoot length (cm)	Mean No. of tillers	Mean No. of leaves
Effects of varieties			
HSF-240	10.50 a	5.400 a	17.50 a
CP-77-400	5.250 b	3.600 a	11.60 b
CPF-237	6.700 b	3.500 a	9.800 b
Effects of Hormones combinations			
T_1	7.500 b	3.500 b	9.500 b
T_2	9.167 ab	4.833 ab	16.00 a
T_3	10.17 a	6.167 a	18.83 a
T_4	9.333 ab	6.167 a	18.67 a
T_5	1.250 c	0.1670 c	1.833 c
Interaction effects of varieties and hormones combinations			
HSF-240 × T_1	9.500 cde	3.500 cde	12.00 cd
HSF-240 × T_2	11.00 bcd	4.500 bcd	19.00 bc
HSF-240 × T_3	14.50 ab	8.000 ab	22.00 b
HSF-240 × T_4	16.50 a	11.00 a	32.50 a
HSF-240 × T_5	1.000 h	0.0000 e	2.000 ef
CP-77-400 × T_1	6.50 ef	3.500 cde	8.500 def
CP-77-400 × T_2	4.500 fgh	4.000 bcde	10.50 cdef
CP-77-400 × T_3	8.500 cdef	7.000 abc	24.50 ab
CP-77-400 × T_4	5.500 efg	3.500 cde	12.50 cd
CP-77-400 × T_5	1.250 h	0.000 e	2.000 ef
CPF-237× T_1	6.500 ef	3.500 cde	8.000 def
CPF-237× T_2	12.00 bc	6.000 bc	18.50 bc
CPF-237× T_3	7.500 def	3.500 cde	10.00 cdef
CPF-237× T_4	6.00 ef	4.000 bcde	11.00 cde
CPF-237× T_5	1.500 gh	0.5000 de	1.500 f

Legends:

T_1 = 0.5 mg/l BAP + 0.1 mg/l Kin, T_2 = 1.0 mg/l BAP + 0.1 mg/l Kin, T_3 = 1.0 mg/l BAP + 0.5 mg/l Kin, T_4 = 1.5 mg/l BAP + 0.5 mg/l Kin, T_5 = 0.0 mg/l BAP + 0.0 mg/l Kin

Table 2a. Initiation of sugarcane varieties at 1.0 mg/l Kin and 0.1 mg/l GA_3.

Varieties	Percentage growth	Shoot length (cm)	Leaves no./ plant
HSF-240	85 a	9.0 a	7.0 a
CP-77-400	70 bc	7.5 abc	4.0 bcde
CPF-237	80 ab	8.0 ab	6.0 ab

Table 2b. Effects of different concentrations of IBA on roots induction in three sugarcane varieties.

IBA Conc. (mg/l)	HSF-240		CP-77-400		CPF-237	
	Means roots number	Means root length (cm)	Means roots number	Means root length (cm)	Means roots number	Means root length (cm)
0.5	35a	3.05a	20.5d	2.05a	10.5g	1.3de
1.0	23c	1.75c	41a	1.55b	15.5f	1.2ef
1.5	21cd	2.05b	14.5ef	1.25cd	34.5a	1.8a

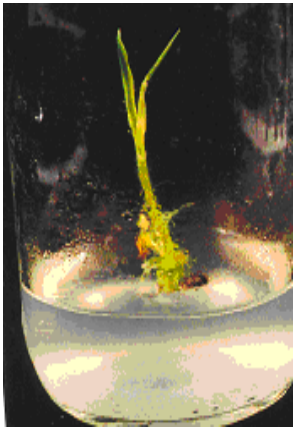

Fig. 1. *In vitro* shoot tip growth of var. HSF-240 at 1.0 mg/l Kin + 0.1 mg/l GA_3.

Fig. 2. *In vitro* shoot tip growth of var. CP-77-400 at 1.0 mg/l Kin + 0.1 mg/l GA_3.

Fig. 3. *In vitro* shoot tip Growth of var. CPF-237 at 1.0 mg/l Kin + 0.1 mg/l GA_3.

Fig. 4. *In vitro* shoot multiplication of var. CP-77-400 at 1.0 mg/l BAP + 0.5 mg/l Kin.

Fig. 5. *In vitro* shoot multiplication of var. CPF-237 at 1.0 mg/l BAP + 0.1 mg/l Kin.

Fig. 6. *In vitro* shoot multiplication of var. HSF-240 at 1.5 mg/l BAP + 0.5 mg/l Kin.

Fig. 7. *In vitro* rooting of var. HSF-240 at 0.5 mg/l IBA.

Fig. 8. *In vitro* rooting of var. CP-77-400 at 1.0 mg/l IBA.

Fig. 9. *In vitro* rooting of var. CPF-237at 1.5 mg/l IBA.

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