INFLUENCE OF GROWTH REGULATORS ON MULTIPLICATIONS OF SUGARCANE (*SACCHARUM OFFICINARUM.* L) VARIETY CO 6886

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ABSTRACT

Sugarcane (Saccharum officinarum L.) is a member of the genus Saccharum from family Gramineae and is an important agricultural cash crop in tropical and subtropical region of the world. The objective of this study was to evaluate the effect of different concentration of cytokinins (BAP, kinetin and 2iP) and auxins (IBA, and NAA) combinations of different plant growth regulators on the sugarcane cv. Co 6886.

Plant material of sugarcane cv. Co 6886 was selected form greenhouse in Plant Tissue Culture Laboratory at Agriculture Research Corporation (ARC) in Sudan. The young meristem cutting explants were inoculated on to sterilized solid basal MS medium (Murashige and Skoog's, 1962) supplemented with different concentrations of cytokinins BAP (1.0 - 2.5mg/l), kinetin (1.0 - 2.5mg/l) and 2iP (1.0 - 2.5mg/l) and concentration of auxins (IBA (2.0 - 5.0mg/l) and NAA(2.0 – 5.0mg/l) on combinations of different plant growth regulators.

Data were collected after two weeks, four weeks and six weeks to evaluate the morphogenesis of shoot-tip explant and evaluate the numbers of roots per explant which have effect on plant growth regulators on the sugarcane cv. Co 6886.

Result indicates that the explant generated from shoot-tip was highest on MS medium supplemented with BAP (2.0 – 2.5mg/l), Kinetin (1.0 – 2.5mg/l) and 2iP (2.0 – 2.5mg/l) all of them after two, four and six weeks. While the rooting were observed on MS medium supplemented show the best with the IBA concentrations (5.0mg/l) and NAA (2.0 - 5.0mg/l) after two, four and six weeks.

Keyword: Auxins Cytokinins and Sugarcane *Saccharum officinarum* L Co6886

1. INTRODUCTION

Sugarcane (*Saccharum officinarum* L.) is a member of the genus *Saccharum* from family Gramineae and is an important agricultural cash crop in tropical and subtropical region of the world and is the major source of sugar with respect to export product in many developing countries that accounts for more than 60% of the world's sugar production (Guimarces and Sobral, 1998). There are many causes of low yield, one of which is the lack of a rapid seed multiplication procedure. Once a desired clone is identified, it usually takes 6-7 years to produce sufficient quality of improved seed material. This long duration causes a major bottleneck in breeding programmers (Siddiqui et al., 1994). Another important reason for low yield in sugarcane is its susceptibility to attacks by pathogens such as fungi, virus, bacteria and mycoplasma which cause up to 70% in yields reduction (Xue & Chen., 1994; Oropez et al., 1995; Bhavan & Gautam, 2002). During the last thirty years, micro propagation and other *in vitro* techniques have become more widely used in commercial horticulture and agriculture for the mass propagation of crop plants (George and Sherrington, 1984; Dodds, 1991; George, 1993; Das et al., 1996).In sugarcane, micropropagation is important for rapid multiplication of elite genotypes/clones and for the quick spread of new varieties (Nickell and Heinz, 1973). Tissue culture of sugar-cane has received considerable research attention because of its economic importance as a cash crop. Plant regeneration through tissue culture technique would be a viable alternative for improving the quality and production of sugar-cane. Initial attempts to regenerate plants through in vitro technique were made on sugar-cane by Nickell (1964) and Heinz and Mee (1969). In sugarcane conventional propagation is through sets which is slow, usually one to ten in a period of one year. Moreover, pathogens keep on accumulating generation after generation which reduces the yield and quality of sugarcane. Usually due to lack of multiplication procedures, it requires 10 - 15 years to complete the selection cycle and to get an improved variety for commercial cultivation. The time spent for this multiplication is considered 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 required for mass multiplication. Micropropogation through tissue culture holds immense potential for mass multiplication and subsequent rejuvenation and quality production (Heinz and Mee, 1969). Protocols for *In vitro* plant regeneration of sugarcane through callus culture, axillary bud and shoot tip culture have been developed by many authors (Lee 1986, 1987; Hu & Wang 1983; Hendre et al., 1983; Milton & Alien 1995; Baksha et al., 2002). One of the major obstacles to the In vitro micropropagation of plants is the genotype / media interaction and rooting of the plantlet. Sugarcane is a highly heterozygous, polyploid and aneuploidy crop (Jannoo et al., 1999) and as a consequence the frequency of shoot differentiation from apical shoots in most sugarcane varieties varies greatly in number (Siddiqui et. al., 1994). Standardization of protocols for in vitro multiplication of sugarcane through callus culture, axillary bud and shoot tip culture have been reported by many authors (Barba et al.,1978 ; Nadar et al.,1978 ; Bhansali and Singh, 1984 ; Anita et al., 2000). However, reports are scare on young meristem shoot culture in sugarcane cultivar, *cv.* Co 6886. The present communication demonstrates an effective high frequency regeneration method which allows for expedient multiplication of micro plants that are easily established ex vitro through shoot-tip culture of young meristem as an explant. Therefore the objective of this study was to evaluate the effect of different concentration of cytokinins (BAP, kinetin and 2iP) and auxins (IBA, and NAA) combinations of different plant growth regulators on the sugarcane *cv.* Co 6886 selected form greenhouse in Plant Tissue Culture Laboratory at Agriculture Research Corporation (ARC) in Sudan.

2. MATERIALS AND METHODS:

2.1 Plant Material:

The plant material of sugarcane *cv.* Co 6886 was selected form greenhouse in Plant Tissue Culture Laboratory at Agriculture Research Corporation (ARC) in Sudan.

2.2Culture medium:

The young meristem cutting explants were inoculated on to sterilized solid basal MS medium (Murashige and Skoog's, 1962) supplemented with different concentrations and combinations of different plant growth regulators.

2.2.1 Effect of different concentrations of cytokinins on shoot-tip:

This experiment was conducted to test the effect of different concentrations of cytokinins as following BAP (1.0, 1.5, 2.0, 2.5mg/l), kinetin (1.0, 1.5, 2.0, 2.5mg/l) and 2iP (1.0, 1.5, 2.0, 2.5mg/l on the sugarcane *cv.* Co 6886. Data were collected after two weeks, four weeks and six weeks to evaluate the morphogenesis of shoot-tip explant.

2.2.2 Effect of different concentrations of auxins on explant:

This experiment was conducted to test the effect of different concentrations of auxins as following IBA (2.0, 2.5, 3.0, 5.0mg/l) and NAA (2.0, 2.5, 3.0, 5.0mg/l) on the sugarcane *cv.* Co 6886. Data were collected after two weeks, four weeks and six weeks to evaluate the numbers of roots per explants.

3. RESULTS AND DISCUSSION:

3.1 Effect of different concentrations of cytokinins on shoot-tip:

The number of shoots regenerated per explants increased significantly with increase of BAP concentration. Table (3.3.1.1) shows the percentages of explants with shoot morphogenesis significant higher number of shoots per explants was induced on MS medium supplemented with BAP (1.5, 2.0 - 2.5mg/l) after two and four weeks compared with MS medium. After six weeks all BAP concentration induced significantly high no of shoot compared with MS medium. No significant different were observed between all BAP concentration after six weeks. These results are in contrast to the report of Alam *et al*. (1995) where 0.5-1 mg/l BAP were used for shoot multiplication. The study of Anon. (1985) indicated that normal shoot initiation and development was obtained in two cultivars CO-62171 and CO-7201 with BAP 0.05 mg/l only. These observations suggest that concentration of BAP required for shoot initiation and establishment varies with genotypes. The amount of cytokinin applied and found adequate depends on the genotype used and the micropropagation strategy employed. Most investigators prefer to secure proliferation of shoots along with normal development of shoot from the cultured bud or the meristem. In such cases higher cytokinin levels have been used, whereas, normal development of the shoot from the bud meristem might require very low levels of the growth regulators, as in case of Sreenivasan and Jalaja (1983).

Table (3.3.1.1): Effect of different concentrations of BAP on shoot-tip of sugarcane *cv.* Co. 6886:

Treatment	**Number of shoots per explant**		
BAP mg/L	Two weeks	Four weeks	Six weeks
0.0	1.3 b	1.6 b	1.9 b
1.0	1.5 ab	2.0 bc	3.4 a
1.5	1.7 a	2.3 a	3.9 a
2.0	1.8 a	2.4 a	4.1 a
2.5	1.7 a	2.3 a	4.1 a
SE±	0.04	0.06	0.14
C.V %	15.5	16.4	15.5

Table (3.3.1.2) shows the percentages of explants with shoot morphogenesis and number of shoots regenerated per explants increased significantly with increase of kinetin concentration. There was significant differences no of shoots per explants induced on MS medium devolved from kinetin (1.0, 1.5, 2.0, 2.5mg/l) and MS medium after two, four and six weeks compared with kinetin concentrations. All kinetin concentration was comparable in no of shoot per explant. Cheema and Hussain (2004) observed 29 shoots per plant at 0.4 mg/l BAP in combination with 0.4 mg/l Kin. This strongly supports the use of cytokinin for multiple shoot formation but we recorded low level of cytokinin compared to them.

Table (3.3.1.2): Effect of different concentrations of Kinetin on shooting-tip of sugarcane *cv.* Co. 6886:

Treatment	**Number of shoots per explant**		
Kinetin mg/L	Two weeks	Four weeks	Six weeks
0.0	1.3 b	1.5 b	1.9 b
1.0	1.7 a	2.1 a	3.9 a
1.5	1.7 a	2.2 a	3.8 a
2.0	1.7 a	2.3 a	3.9 a
2.5	1.7 a	2.1 a	3.8 a
SE±	0.04	0.07	0.13
C.V %	16.5	19.9	14.3

Table (3.3.1.3) shows the percentages of explants with shoot morphogenesis and number of shoots regenerated per explants increased significantly with increase of 2iP concentration. There was significant differences in no of shoots per explants was induced on MS medium devolved from 2iP and MS medium after two, four and there was higher significant of number of shoots per explants after six weeks compared with 2iP concentration after two and four weeks.

Table (3.3.1.3): Effect of different concentrations of 2ip on shoot-tip of sugarcane cv. Co.6886:

Treatment	Number of shoots per explant		
2iP mg/L	Two weeks	Four weeks	Six weeks
0.0	1.3 a	1.8 a	2.1 b
1.0	1.5 a	2.0 a	2.3 b
1.5	1.5 a	2.3 a	2.8 ab
2.0	1.7 a	2.2 a	3.1 a
2.5	1.5 a	1.9 a	2.5 ab
SE±	0.05	0.07	0.09
C.V	20.8	24.4	21.8

3.2. Effect of different concentrations of auxins on explant:

The number of roots regenerated per explants increased significantly with increase of IBA concentration. Table (4.3.2.1) shows the percentages of explants with roots morphogenesis number of roots per explants induced was significant higher on MS medium with IBA at 5.0mg/l after two, four and six weeks compared with other IBA concentrations the number of roots induced by 5.0 mg/l IBA was significantly higher than other concentration after six weeks. Sabaz et al., (2008) used 1.0 mg/l IBA as the best root initiating growth hormone with highest number of 41 roots per plant. Ali and Afghan (2001) observed only 6 - 7 roots after 3 weeks on MS medium containing 2.0 mg/l IBA and 6% sucrose. These findings also agree well with the previous findings of Nadar and Heinz (1977). Alam et al., (2003) reported best rooting response at 2.5 mg/l IBA with 16 number of roots/explant having 1.1 cm root length.

Table (4.3.2.1): Effect of different concentrations of IBA on explant of sugarcane *cv.* Co. 6886

Treatment	Number of root per explant		
IBA mg/L	Two weeks	Four weeks	Six weeks
0.0	1.6 b	1.8 b	2.3 b
2.0	1.2 c	1.8 b	2.4 b
2.5	1.8 ab	1.9 b	2.6 b
3.0	1.9 ab	2.1 ab	2.6 b
5.0	2.1 a	2.5 a	3.1 a
SE±	0.05	0.06	0.06
C.V %	14.3	16.2	13.1

Table (4.3.2.2.) shows the percentages of explants with roots morphogenesis and number of roots regenerated per explants was significantly higher on all NAA concentrations compared with MS medium devoted from NAA concentration. There were no significant differences on number of roots regenerated per explants on all NAA (2.0, 2.5, 3.0,5.0 mg/l) after two, four and six weeks. Baksha et al., (2002) used 5.0 mg/l NAA for best rooting response in half strength MS medium. Many workers also reported that 5 mg/l NAA was good for rooting (Larkin, 1982, Shukla *et al.*, 1994,Alam *et al*., 1995, Islam *et al*., 1996) and more than 5 mg/l NAA inhibits rooting. The concentration of hormone varies with variety to variety.

Table (4.3.2.2): Effect of different concentrations of NAA on explant of sugarcane *cv.* Co. 6886

Treatment	**Number of root per explant**		
NAA mg/L	Two weeks	Four weeks	Six weeks
0.0	1.6 b	1.6 b	2.1 b
2.0	1.9 a	2.9 a	3.0 a
2.5	2.0 a	2.7 a	3.1 a
3.0	2.0 a	2.4 a	3.0 a
5.0	2.1 a	2.5 a	3.0 a
SE±	0.05	0.08	0.08
C.V %	14.9	14.8	13.4

4. CONCLUSION:

Micropropagation of sugarcane (*saccharum officinarum.*) from shoot tip may become the successful method with the present day demand. It will be an easy way for obtaining intensive number of plants in limited time under controlled conditions. the use of tissue culture technique it may be easy to obtain disease free plants. The protocol used in the present study can be used for rapid multiplication of sugarcane.

5. Acknowledgment:

I give thanks to all how support and help me with this work and thanks also given to almighty God for save me during preparation this work.

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