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Effect of adenine sulfate, benzylaminopurine and media forms on propagation of banana (Musa AAA) cv. Gros Michel and plantain (Musa ABB) cv. Cardaba

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ABSTRACT

This study was initiated to test the effect of adenine sulfate, benzylaminopurine (BAP) and media forms on in vitro morphogenesis of banana cv. Gros Michel and plantain cv; Cardaba during the period January, 2001 to May, 2002. The number of plantlets per explant increased significantly on both cultivars cultured on liquid propagation medium with filter paper bridge compared to other forms of media. However, the percentage of explants with shoot regeneration was comparable on all treatments. Comparable percentage of shoot tip explants with shoots were induced from cv. Gros Michel culture on different concentrations of adenine sulfate and the propagation medium which represented the control. However, the number of shoots per explant decreased significantly except that on 100mg/l which was comparable with the propagation medium. The percentage of explants with roots and number of roots per explant increased significantly on cv. Gros Michel on adenine sulfate compared with the propagation medium. The different concentrations of adenine sulfate induced comparable percentage of explants with roots and shoots on cv. Cardaba. However, the number of explants with shoots induced on Cardaba decreased significantly when adenine sulfate was added to the propagation medium and the number of roots per explant increased significantly compared with the control. Benzylaminopurine induced similar percentages of explants with shoot regeneration on cvs. Gros Michel and Cardaba. Significantly higher number of shoots per explants of banana cv. Gros Michel was induced on mediun supplemented with BAP at 5 or 7.5 mg/l. However, the highest nurnber of shoots regenerated from plantain cv. Cardaba was induced

by higher concentrations of BAP (7.5 and 10.0 mg/l). There were genotypic differences in the response of the two cultivars to BAP.

INTRODUCTION

Banana and plantain annual world production is estimated to be approximately 88 million tonnes (Anon, 1999), making them one of the largest food crops after rice, maize and wheat. In Sudan, banana fruits are very popular and widely consumed, due to their low price compared with other fruit crops. Dwarf Cavendish is the most widely grown banana cultivar in Sudan with low yield, sensitive to low temperature and is less suitable for export. Poor soil fertility, inadequate crop management and postharvest handling are major constraints facing banana production and export. To improve banana production for both internal market and export, there is a need to introduce and test new cultivars to meet quality standards. Furthermore, improvement in production techniques, cultural practices and postharvest handling is urgently needed.

Sudanese farmers use suckers from neglected orchards which might be infected with pests such as nematodes (Hamid, 1992). Also, the conventional method of propagation does not offer a quick mean to supply planting material of newly released cultivars. Micropropagation of banana provides the capacity to deliver •large numbers of plants at planting time and location with great practical and economic benefit. The main advantages of plant tissue culture over conventional suckers are mass propagation of pathogen-free planting material, production of uniform and true to type planting material.

Wargantiwar et al. (1997) demonstated that the maximum multiple shoot regeneration from banana was produced using 7 mg/l BAP or 5 mg/l BAP plus 15% coconut milk, and reported that the highest number of shoots were regenerated from shoot tip of banana cv.Shrimanti on Murashige and Skoog's medium (MS) basal salts (Murashige and Skoog, 1962) supplemented with 7 mg/l BAP. For Musa shoot tip culture, only auxins and cytokinins were required (Vuylsteke, 1989). Stimulation of multiple shoots or bud formation was achieved by culturing explants on media supplemented with relatively high levels of cytokinin (Vuylsteke, 1989). This reduced the dominance of the apical meristem causing adventitions and /or

axillary buds to grow. directly from the explant. The most Widely used and most effective cytokinin for this purpose is BAP, which has been found to be superior to kinetin (Cronauer and Krikorian, 1984; Wong,1986; Zamora et al., 1986).

Fitchet and Winnaar (1988) reported that the best development of banana explant was obtained when shoot tips of the .cvse Dwarf Cavendish and Williams were cultured on mixture of IBA (2 my/l), NAA (2 mg/l) kinetin (5 mg/l), adenine sulfate (160 mg/l), sodium phosphate (34 mg/l) and activated charcoal (5 g/l). Ahsan et al. (2000) found that the best regeneration of roots in the proliferated shoots of banana (Musa sappientum) variety Sagar was obtained on MS medium supplemented with 40,0 mg/l adenine sulfate plus 7.0 mg/l dihydrogen phosphate, Rapid mass micropropagation of banana cvs. Williams and Grand Naine was investigated by Bekheet and Saker (1999). They found that addition of adenine sulfate to the multi-plication medium increased the number of proliferated shoots, but decreased the number of leaves and shoot length. Vani and Reddy (1999) reported that the highest shoot proliferation of different banana cultivars was induced on MS medium supplemented with 4 mg/l BAP plus 2 mg/l IAA without adenine sulfate.

The objective of this study is to investigate the effect of benzylam-inopurine (BAP), adenine sulphate and different forms of nutrient media on commercial propagation of newly introduced cultivars of banana and plantain.

MATERIALS AND METHODS

Sword suckers from the dessert banana cv. Gros Michel and plantain cv. Cardaba were collected from a greerülouse at the Agricultural Research Corporation, Wad Medani, Sudan during the period January, 2001 to May, 2002. Shoot tips were surface sterilized by shaking in 50% (v/v) commercial bleach (Clorox) with two drops of Tween 20 for 30 minutes and washed 3 times with sterilized distilled water. Murashige and Skoog's medium (MS) basal salts (Murashige and Skoog, 1962) was used. The pH of the medium was adjusted to 5.7 ± 0.1 prior to addition of the gelling agent (Gelrite, Sigrna) and then sterilized for 15 minutes at 1.05 kg/cm2 and •121 oc. The morphogenetic response of shoot tip explants of the banana cv.

Gros Michel (AAA' and plantain cv. Cardaba (ABB), was tested or MS medium supplemented with 0.175 mg/l indole acctic acid (IAA) and different concentrations of BAP (0, 2.5, 5, 7.5 and 10 mg/l). In addition, response of the two cultivars of Musa sp. was tested on MS medium supplemented with different concentrations of adenine sulfate (0, 50, 100, 150, 200 mg/l), and different3 forms of propagation medium (semisolid, liquid, liquid plus filter paper, liquid plus cotton).

The cultures were incubated at 27 ^{o}c for 16 h photoperiod and the light intensity was 1500 Lux. Data was collected after four and eight weeks and included the number of explants with shoots and roots, number of shoots and roots per explant and plant height, from 10 replications per treatment in a completely randomized design. Statistical analysis of the data was done by MstatC microcomputer programme and means were separated according to Duncan's Multiple Range Test (MSU, 1993).

RESULTS AND DISCUSSION

The different forms of propagation media (MS supplemented with 0.175 mg/l IAA plus 2.2 mg/l BAP) were simillar on percentage of shoot morphogenesis of banana cv. Gros Michel and plantain cv. Cardaba (Table 1). Explants of both banana and plantain cultured on

Table 1. Percentage of explants with shoot and number of shoots per explant in banana cv. Gros Michel and Plantain cv. Cardaba on different forms of propagation media after 4 weeks.

Propagation medium	Explants with shoots (%)		No. of shoots per explant	
	Gros Michel	Cardaba	Gros Michel	Cardaba
Liquid	80.0	100	1.5 c	5.3 b
Liquid +Filter paper	100.0	100	4.5 a	9.9 a
Liquid +Cotton	100.0	100	3.5 b	5.5 b
Semi-solid (agar) medium	90.0	100	1.9 c	3.9 c
Mean	92.5	100	2.9	6.2
cv (0/0)	19.0	O	18.4	10.4

Means followed by different letters in the same column were significantly different at 1% probability level using the Least Significant Difference test.

liquid medium with filter paper bridge had significantly higher multiplication rate compared with other forms of nutrient media (Table l), The number of shoots regenerated from banana cv. Gros Michel on liquid medium with cotton bridge was the second best amongst other forms of propagation media, When both cultivars were cultured on semi-solid medium, siY1ificantly lower number of shoots per explant were induced (Table 1).

Different concentrations of adenine sulfate in the propagation medium induced comparable percentages of shoot morphogenesis of banana cv. Gros Michel after 8 weeks (Table 2). There were no significant differences between the propagation media supplemented with 50, 150 and 200 mg/l adenine sulfate on the number of shoots regenerated per explant of cv. Gros Michel except 100 mg /l which was comparable with the propagation medium (Table 2). Shoot tip explants of the plantain cv. Cardaba incubated on the propagation medium without adenine sulfate gave comparable percentage of explants with shoots with all concentrations of adenine sulfate. However, significantly higher number of shoots per explant were induced on this medium compared with those supplemented with adenine sulfate, The number of shoots decreased significantly with the increase in concentration of adenine sulfate (Table 3).

Table 2. In vitro morphogenesis of banana cv. Gros Michel on MS medium supplemented with different concentrations of adenine sulfate after 8 weeks.

Adenine sulfate (mg/l)	Explants with shoot (%)	No. of shoots per explant	Explants with root (%)	No. of roots per explant	Explant height (cm)
0	100	3.1 a	50.0 b	0.4 C	2.1 c
50	100	1.4 b	90.0 a	2.4 a	2.3 b
100	100	3.6 a	80.0 a	0.4 c	2.6 a
150	100	1.8 b	100.0 a	2.1 b	2.7 a
200	100	1.7 b	90.0 a	2.3 ab	2.5 ab
Mean	100	2.3	82.0	1.5	2.4
cv (0/0)	0	16.9	22.8	14.9	10.2

Means followed by different letters in the same colurnn were significantly different at 1% probability level using the Least Significant Difference test.

Table 3. In vitro morphogenesis of plantain cv. Cardaba on propagation medium suppplemented with different concentrations of adenine sulfate after 8 weeks,

Adenine sulfate (mg/I)	Explants with shoots (%)	No. of shoots per explant	Explants with roots (%)	No. of roots per explant	Explant height (cm)
0	100	1.9 a	80.0	2.1 bc	2.5 e
50	100	1.3 c	90.0	2.5 b	3.2 d
100	100	1.5 b	100.0	2.7 b	4.1 c
150	100	1.0 d	100.0	3.8 a	6.3 a
200	100	1.1. d	90.0	3.8 a	5.9 b
Mean	100	1.4	92.0	3.0	4.4
CV(%)	0	13.4	17.2	4.7	6.0

Means followed by different letters in the same column were significantly different at 1% probability level using the Least Significant Difference test.

The percentage of explants with roots in banana cv. Gros Michel was significantly lower on medium supplemented with adenine sulfate compared with adenine sulfate free medium (Table 2). All concentrations of adenine sulfate ranging from 50 to 200 mg/l were comparable in the frequency of root morphogenesis of the banana cv. Gros Michel (Table 2). In contrast, the percentage of roots per explant of cv. Cardaba on propagation medium supplemented with different concentrations of adenine sulfate were comparable (Table 3), The number of roots per explant regenerated from banana cv. Gros Michel varied significantly amongest the different concentrations of adenine sulfate. Significantly higher number of roots per explant of banana cv. Gros Michel was found on propagation medium supplemented with 50 mg/l adenine sulfate compared with other concentrations, except 200 mg/l. The number of roots per explant regenerated from plantain cv. Cardaba was significantly higher on propagation medium supplemented with 150 and 200 mg/l adenine sulfate compared with other concentrations.

Plantlet height of banana cv. Gros Michel increased significantly with the increase in adenine sulfate. Plantlet height of banana cv. Gros Michel was comparable on propagation medium supplemented with 100,150 and 200 mg/l adenine sulfate. The plant height of plantain cv. Cardaba decreased significantly at low concentrations of adenine sulfate.

The percentage of explants with shoots of banana cv. Gros Michel and plantain cv. Cardaba was comparable one MS medium supplemented with 00175 mg/l IAA and different concentrations of BAP after 8 weeks (Table 4). The number of shoots per explant increased significantly with the increase in BAP concentration from 0 to 5.0 mg/l on banana cv. Gros Michel, and from 0 to 7.5 mg/I BAP on plantain cv. Catdaba. Significantly higher number of shoots per explant of banana cv.- Gros Michel was obtained either at 5.0 or 7.5 mg/l BAP compared with other concentrations of BAP, whereas siY1ificantly higher number of shoots per explant of plantain cv. Cardaba was obtained at 7.5 and 10 mg/l BAP compared with other concentrations.

Table 4, In vitro morphogenesis of banana cv. Gros Michel and plantain cv. Cardaba on MS medium supplemented with BAP after 8 weeks.

BAP (mg/l)	Percentage of explants with shoot		No. of shoots per explant	
	Gros Michel	Cardaba	Gros Michel	Cardaba
0.0	80	100	0.8 d	0.5 c
2.5	100	100	1.9 c	1.8 b
5.0	100	100	3.3 a	1.6 b
7.5	100	100	3.4 a	4.1 a
10.0	90	100	2.5 b	3.7 a
Mean	94	100	2.4	2.3
cv (0/0)	19.1	0	17.1	19.3

Means followed by different letters in the same colunn were Significantly different at 1% probability level using the Least Significant Difference test

Liquid medium was found to be the best for microprpagation when cultures were supported b} filter paper due to the availability of nutrients in the medium to the explants. This result is consistent with Murashige (1977) who used filter paper to support the tissue and improve aeration of stationary liquid cultures. Contrary to the results of this study, adenine sulfate reported by Vani and Reddy (1999) to induce higher multiplication rates on different cultivars of banana. Genotypic variation 'in response to adenine sulfate was reported by Banerjee and Sharma (1988). The inconsistency in the response of

both banana genotypes to adenine sulfate was possibly caused by the presence of other growth regulators in the propagation medium which were more effective in the induction of shoot regeneration and suppression of rooting as shown by the control.

The best propagation of both genotypes of banana was found between 5 and 10 mg/l. The differences in the morphogenetic response of the two banana cultivars in the number of shoots per explant indicates genotypic variation These results were consistent with other authors who used 5-7 mg/l BAP for micropropagation of banana (Cronauer and KHkorian, 1984; Zamora et al., 1986; Vuyl-steke, 1989; Wargantiwar et Benzylaminopurine concentr- ation above 10 mg/l was reported by Wong (1986) to reduce the rate of shoot multiplication. There was genotypic variation in the response to BAP. The differences in in vitro morphogenesis among banana genotypes was reported by Das et al, (1998). Vuylsteke 1989) reported that proliferation and multiplication of banana depended on genotype and culture medium composition.

In coclusion, the microprpagation of the banana cvs, Gros Michel and Cardaba can be achieved on 5 and mg/l of BAP, respectively, and liquid medium with filter paper is the best medium for micropro-pagation of banana. Adenine sulfate was inconsistant in its effect on micropropagation of banana cultured on MS medium supplemented with BAP and IAA.

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