Effect of different cytokinins concentrations and carbon sources on shoot proliferation of bitter almond nodal cuttings

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Abstract: The present work was carried out in plant tissue culture Laboratory, Agriculture Development Systems project (ADS) at Giza, Egypt during the period from 1999-2003. It aimed to study the effect of various cytokinins at different concentrations (0.0, 0.5, 1.0, 2.0 and 4.0 mg/L) and effect of carbon source (Sucrose, Glucose and fructose) and their concentrations on shoot number and shoot length /explant and fresh weight (g/explant0 on bitter almond nodal cuttings. The most effective cytokinine for enhancing in vitro growth was BA followed by kinitine and zeatin respectively. Lower concentration of BA and kinitine at (0.5 and 1.0 mg/L) gave healthier plants than 2.0 or 4.0 mg/L. However, using 4.0 mg/ L BA in shoot proliferation medium increased in vitro growth (No.of shoots /explant, fresh weight) of bitter almond nodal cuttings. Glucose was the most effective carbon source for stimulating the production of shoots, fresh weight and shoot length on the new developed shoots. It was followed by sucrose and fructose. On the other hand, sucrose gave healthier plant than glucose or fructose. [Journal of American Science. 2010;6(9):465-469]. (ISSN: 1545-1003).

Keywords: cytokinins, carbon sources**,** shoot proliferation, nodal

1. Introduction

Almond is a species difficult to propagate either by cuttings (Hartmann et al, 1990) or in vitro by micropropagation(Rugini and Verma 1983) .The best shoot multiplication of almond cv. Ferraduel, walnut (unknown ecotype) and chestnut was obtained on DKW medium supplemented with (3.5 µM BA and 0.08 µM NAA,), (4.5µM BA and 0.05 µM IBA) and 1 µM BA respectively (Rugini et al 1993). Moreover, using BA at 2.2 µM and 1.4 µM IAA stimulated the multiplication of two almond genotypes (Cultivar Supernova and rootstock M51) (Caboni and Damiano 1994)

The highest proliferation rate for the two almond cultivars (Neplus and Nonpareil) was achieved with 2 mg / L BAP (Saeed 1998). Moreover, BA concentration between 0.5 and 0.6 mg / L produced the optimum number of shoots of a good length of apricot cv. Canino (Perez et al 2000).

The most effective cytokinin for enhancing the production of shoots and leaves from Canino and Amar nodal cutting was BA(at 1-2 mg / L) and it was followed by tip at 2 mg / L and kinetin at 2 mg / L (Kandil 2001).

Sucrose is generally regarded as the best carbon source and is universally used as the principally energy source. However in certain cases, it may be substituted by glucose and fructose but most other sugars are poor carbohydrate sources for the plant sucrose concentrations generally range from 2-4 % (Wetherell 1982).

Sugar is very important component in any medium. Sugar is essential for in vitro growth and development. A concentration of 1.5 % sucrose (a disaccharide) is usually used in in vitro, since this sugar is also synthesized and transported naturally by the plant. Glucose and Fructose may be used. The sugar concentration chosen is very dependent on the type and age of growth material (Pierik 1987).

2. Material and Methods

The present work was carried out during the period between 1999 till 2003 in the laboratories of Agriculture Development System (ADS).

This study aimed to investigate the effect of different cytokinins and their concentrations and different carbon sources on shoot proliferation of bitter almond.

Effect of different cytokinins and their concentrations on shoot proliferation:

In this experiment, different cytokinins (BA, Kin. And Zeatin) and different concentrations (0.0, 0.5, 1.0, 2.0 or 4.0 mg / L) for each were added individually to MS medium which was supplemented with 30 g / L sucrose + 0.1 mg / L IBA + 0.1mg / L GA_3 + 7.0 g / L agar .

Effect of different carbon sources on shoot proliferation:

In this experiment, different carbon sources (glucose, fructose and sucrose) were investigated each sugar was added individually to the MS culture medium at the concentrations of (0, 1 %, 2 %, 3 %, or 4 %).

Culture medium of each treatment was supplemented with 2.0 mg / L BA + 0.1 mg / L IBA + 0.1mg / L GA_3 + 7.0 g / L agar .Explants of each treatment of different experiments were cultured for 6 weeks. The following growth parameters were determined:

a- Number of lateral shoots.
b- Shoot length / explants.
c- Fresh weight.

3. Results and Discussion

Effect of cytokinins and their concentrations

a- Shoot number / explant

Data in Table (1) show the effect of various cytokinins at different concentration (0.0, 0.5, 1.0, 2.0 and 4.0 mg /L) on in vitro growth (shoot number / explant) of bitter almond rootstock. It was clear that BA was the most effective cytokinin to produce the highest significant value of shoot number / explant (24.0) followed by kinetin (Kin.) and zeatin (Zea.) (14.27 and 11.80) respectively .

Regarding the effect of concentrations, it was obvious that 4.0 mg/L gave the highest significant number of shoot / explant (26.0) followed by 2 mg/L (20.33).

Data also revealed a significant interaction between cytokinins and their concentration as BA at 4.0 mg/L produce the highest significant shoot number / explant.

b- Shoot length

The effect of various cytokinins and their concentration on shoot length in cm was presented in Table (2).

It was clear that, kin gave the highest significant shoot length (4.23 in cm.) followed by both BA and Zea. With insignificant differences in between.

However using 0.5 mg/L resulted in the longest explant (4.55cm) compared with other cytokinins concentration.

Significant interaction was obtained between types of cytokinin and their concentrations, as kin at 0.5 mg/L resulted in the longest explant compared with other treatments.

c- Fresh weight

Results in Table (3) present the effect of various cytokinins and their concentrations on fresh weight of bitter almond rootstock. It was noticed that BA resulted in the highest significant fresh weight followed by kin and zea, respectively (4.55, 2.76 and 1.79 g / explant).

Regarding the effect of concentrations, it was clear that, 1.0 and 4.0 mg / L recorded the highest significant fresh weight (3.76 and 3.69 g / explant) respectively .

Data also revealed a significant interaction as BA at 1.0 mg / L gave the highest average of fresh weight / explant (6.66 g / explant).

In general, it could be concluded that, increasing the concentrations of various cytokinins from 0.5 to 4.0 mg / L increased significantly the production of shoot / explant and fresh weight value.

Meanwhile, the longest explants of bitter almond rootstock were obtained on MS medium contained BA at 0.5 mg / L.

However, using lower (0.5 and 1.0 mg / L BA) concentration of BA resulted in more healthy explants.

These results are in agreement with those found by Rugini and Verma (1983); Gunidy (1990); Fouad et al (1995); Sari El-Deen (1998) and Perez et al (2000). They reported that the highest proliferation rate of almond, Nemaguard peach and apricot cv. Canino was observed with BA at low concentration between 0.2 to 1.0 mg / L.

Effect of different carbon source

a- Shoot number / explant

The effect of various carbon source (Sucrose, Glucose and Fructose) and their concentrations on shoot number / explant of bitter almond rootstock are shown in Table (4).

It was clear that, both sucrose and glucose recorded the the highest significant shoot number / explant (39.50 and 40.50) while, fructose took the other way around.

As for the concentration of sugar, it was noticed that 40 g/ L gave the highest significant number of shoots followed by 30 g/L.

Data also revealed a significant interaction between types of sugar and concentrations as sucrose at 40 was the most effective sugar to produce the highest significant shoot number / explant (60.00).

b- Shoot length

Results in Table (5) show the effect of various concentrations shoot length of almond explant.

It is obvious that, the highest significant value of shoot length in cm. (2.70) was obtained by adding glucose tom's medium followed by sucrose (2.33) .Meanwhile; fructose recorded the lowest number of shoots / explant (1.95).

As for sugar concentrations, it was clear that, 30 g / L resulted in the highest number of shoot length in cm. followed by 40 g / L.

Data also revealed a significant interaction between type of sugars and concentrations as glucose at 30 g / L gave the longest explant (3.83 cm.).

c- Fresh weight

The effect of various types of sugars and their concentrations on fresh weight g number are presented in Table (6).

Results revealed that, glucose was the most suitable sugar that resulted in the highest significant values of fresh weight (8.45 g / explant) followed by sucrose and fructose.

As for the concentrations, it was noticed that higher sugars concentrations (30 and 40 g / L) recorded the highest significant values of fresh weight of bitter almond explant compared with other concentrations.

Significant interaction was obtained between types of sugar and concentrations as glucose at 30 g / L gave the highest significant number of fresh weight.

In general, it could be concluded that glucose at 30 g / L gave the highest number of fresh weight and the longest explant compared with other treatments. However, glucose at 40 g / L gave highest significant shoot number / explant .

On the other hand, sucrose gave healthier explant .Thus sucrose is most suitable sugar that gave the most healthier plant than other sugar types.

These results are in line with those found by Wetherall (1982) and Borkowaska and Szczebra (1991) .They reported that, both sucrose and glucose favored and gave similar rate of proliferation on sour cherry and peach. Also Kandil (2001) stated that, glucose was the most effective carbon source for stimulating the production of Canino and Amar apricot cvs.

Table (1): Effect of different cytokinins and their concentrations on in vitro growth (number of shoot) developed from bitter almond nodal cuttings

Cytokinine Types	Cytokinine concentrations (mg/L)					Mean
	0.0	0.5	1.0	2.0	4.0	
BA	8.33h	17.00ef	26.67c	31.67b	36.33a	24.00A
Kin.	8.33h	12.00g	15.67f	17.00ef	18.33e	14.27B
Zea.	8.33h	7.33h	7.66h	12.33g	23.33d	11.80C
Mean	8.33h	12.11D	16.67C	20.33B	26.00A	

Means having the same letters in column are not significantly different at 5% level

Table (2): Effect of different cytokinins and their concentrations on in vitro growth (shoot length / explant) developed from bitter almond nodal cuttings

Cytokinine Types	Cytokinine concentrations (mg/L)					Mean
	0.0	0.5	1.0	2.0	4.0	
BA	4.16c	4.33c	3.33de	31.67b	36.33a	24.00A
Kin.	4.16c	6.83a	5.83b	17.00ef	18.33e	14.27B
Zea.	4.16c	2.50f	2.23fg	12.33g	23.33d	11.80C
Mean	4.16B	4.55A	3.80C	20.33B	26.00A	

Means having the same letters in column are not significantly different at 5% level

Table (3): Effect of different cytokinins and their concentrations on fresh weight (g) / Explant of bitter almond nodal cuttings

Cytokinine Types	Cytokinine concentrations (mg/L)					Mean
	0.0	0.5	1.0	2.0	4.0	
BA	1.98de	3.60c	6.66a	5.16b	5.33b	4.55A
Kin.	1.98de	2.22d	3.00c	3.20c	3.40c	2.76B
Zea.	1.98de	1.54e	1.61e	1.52e	2.30d	1.79C
Mean	1.98D	2.45C	3.76A	3.29B	3.69A	

Means having the same letters in column are not significantly different at 5% level

Table (4): Effect of different carbon sources and their concentrations on number of shoots developed from bitter almond nodal cuttings

Sugar Types	Sugar concentrations (g / L)				Mean
	10	20	30	40	
Sucrose	23.67cd	26.00cd	48.33b	60.00a	39.50A
Glucose	25.00cd	32.00c	50.00b	55.00ab	40.50A
Fructose	16.67d	21.00d	24.33cd	25.67cd	21.92B
Mean	21.78C	26.33C	40.98B	46.89A	

Means having the same letters in column are not significantly different at 5% level

Table (5): Effect of different carbon sources and their concentrations on number of shoots length (cm.) developed from bitter almond nodal cuttings

Sugar Types	Sugar concentrations (g / L)				Mean
	10	20	30	40	
Sucrose	1.66d	2.16c	2.66b	2.83b	2.33B
Glucose	1.66d	2.83b	8.83a	2.50bc	2.70A
Fructose	1.50d	1.66d	2.16c	2.50bc	21.95C
Mean	1.61D	2.22C	2.88A	2.61B	

Means having the same letters in column are not significantly different at 5% level

Table (6): Effect of different carbon sources and their concentrations on fresh weight (g/explant) on bitter almond nodal cuttings

Sugar Types	Sugar concentrations (g / L)				Mean
	10	20	30	40	
Sucrose	2.08f	2.76def	10.80b	11.08b	6.68B
Glucose	2.87def	4.23de	13.12a	13.60a	8.45A
Fructose	2.11f	2.50ef	4.50d	6.61c	3.92C
Mean	2.35B	3.16B	9.47A	10.09A	

Means having the same letters in column are not significantly different at 5% level

References

1. Borkowaska, B. and J. Szczebra (1991). Influence of different carbon sources on invertaes activity and growth of sour cherry (prunus cerasus L.) shoot culture.J.Exp. Bot., 42: 911-915. (Hort.Abst. 63: 922).
2. Caboni, E. and C. Damiano (1994). Rooting in two almond genotypes. Plantscience, 96:163 – 165.
3. Fouad, M. M.; A. H. Gomaa and M. H. Abd El-Zahar (1995). Factors influencing rooting of peach shoots cultured in vitro. Acta Hort. 409: 197-202.
4. Gunidy, L. F. (1990). Production of some fruit rootstocks through tissue culture technique Ph. D., Fac. Of Agrc., Ain Shams Univ., Egypt.
5. Hartmann, H. T. and D. E. Kester (1990). Plant propagation, principles and practices Fifth Ed. Prentice-Hill, INC Englewood Cliffs, New Jersey, USA.
6. Kandil, E. Abd El-Rahman (2001). Studies on vegetative propagation of Apricot. Ph.D.Thesis, Fac. Of Agric. Cairo Univ., Egypt.

7. Perez, T. O.; J. M. Lopez; L. Egea and L. Burgos (2000). Effect of basal media and growth regulators on the in vitro propagation of apricot (Prunus armeniaca L.)cv. Canino. J. of Hort. and Biotechnology, 75 (3): 283-286.
8. Pierik, R. L. M. (1987). In vitro culture of higher plants. Martinus Nijhoff publisher, Dordrecht, Boston, Lancaster, 1-345.
9. Rugini, E. and Verma (1983). Micropropagation of difficult-to-propagate almond (Prunus amygdalus, batch) cultivar. Plant Sci. Letters, 28: 273-281.
10. Rugini, E.; A. Jacoboni and M. Luppino (1993). Role of basal shoot darkening and exogenous putrescine treatments on in vitro rooting and on endogenous polyamine changes in difficult-to root wood species. Scientia Hort., 53: 63-72.
11. Saeed W. T. (1998). In vitro propagation of two almonds (Prunus dulcis Mill) cv. Bull. Fac. of Agrec.Cairo Univ. 49: 563-574.
12. Sari El- Deen; W. T. Saeed and I. A. Hassabla (1998). Micropropagation of peach rootstocks. Bull. Fac. of Agric.Cairo Univ., 49: 549-562.
13. Wetherall, D. F. (1982). Introduction to in vitro propagation. Selection of culture media Avery publishing group Inc.wayne, New Jersey, USA.

7/10/2010