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Effect of cytokinin type and concentration, and source of explant on shoot multiplication of pineapple plant (*Ananas comosus* 'Queen') *in vitro*

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ABSTRACT

The current study was conducted to test cytokinin type and concentration, and source of explant on shoot multiplication of pineapple plant (*Ananas comosus* 'Queen') *in vitro* during 22/6/2009 to 1/5/2010. The results of the first experiment showed that axillary buds delayed to grow during 4-5 months of culturing in MS medium supplemented with different concentrations of BA (1.0, 1.5, 2.0, 2.5 or 3.0 mg l^{-1}). The MS medium supplemented with 1.0 mg l^{-1} BA has led to the vegetative growth of axillary buds. The other concentrations of BA added to MS medium led to callus growth. The results showed that MS medium supplemented with 1.0 mg l^{-1} BA and 0.2 mg l^{-1} NAA gave adventitious shoots directly after two months from culturing of callus. The results of the second experiment showed that nodal segments cultured in MS medium supplemented with 1.0 mg l^{-1} kinetin was a significantly superior on other treatments studied (0.5 mg l^{-1} kinetin, 0.5 and 1.0 mg l^{-1} BA) in number of shoots/explant, leaf length, number of leaves/shoot and leaf area of formation shoot which reached 18.60 shoot/explant, 5.38 cm, 10.60 leaves/shoot and 3.64 cm^2, respectively. The results showed that kinetin was a significant superior in all vegetative characteristics of shoots compared with BA.

Abbreviation: BA: 6-benzyl adenine.
NAA: α-naphthalene acetic acid.
MS: Murashige and Skoog salts (Murashige and Skoog, 1962)

Key words: *in vitro*, benzyl adenine, axillary buds, kinetin, naphthalene acetic acid, shoot multiplication

IZVLEČEK

UČINEK VRST IN KONCENTRACIJ CITOKININOV TER VIRA STEBELNIH IZSEČKOV NA *IN VITRO* RAZMNOŽEVANJE ANANASA (*Ananas comosus* 'Queen')

Raziskava je bila izvedena z namenom testiranja različnih vrst in koncentracij citokininov ter vira stebelnih izsečkov na *in vitro* razmnoževanje ananasa (*Ananas comosus* 'Queen') v obdobju 22/6/2009 do 1/5/2010. Rezultati prvega poskusa so pokazali, da je rast zalistnih brstov zaostajala prvih 4-5 mesecev gojenja na MS gojišču, ki so mu dodali različne koncentracije BA (1,0, 1,5, 2,0, 2,5 ali 3,0 mg l^{-1}). MS gojišče, ki so mu dodali 1,0 mg l^{-1} BA je vzpodbudilo rast zalistnih brstov. Druge koncentracije BA, dodane MS gojišču so vodile le k rasti kalusa. Nadaljnji rezultati so pokazali, da so se razvili na MS gojišču, ki sta mu bila dodana 1,0 mg l^{-1} BA in 0,2 mg l^{-1} NAA poganjki neposredno iz kalusa po dveh mesecih gojenja. Rezultati drugega poskusa so pokazali, da so bili nodijski izsečki, gojeni na MS gojišču, ki so mu dodali 1,0 mg l^{-1} kinetina značilno boljši kot drugi postopki, izvedeni v tej raziskavi (0,5 mg l^{-1} kinetina, 0,5 in 1,0 mg l^{-1} BA) v številu poganjkov/izseček, dolžini listov, številu listov/poganjek in listni površini nastalih poganjkov. Nastalo je 18,60 poganjkov/izseček, 10,60 listov/poganjek, dolžine 5,38 cm in 3,64 cm^2 površine. Rezultati so pokazali, da je dalo obravnavanje s kinetinom boljše rezultate glede vrednosti vseh merjenih vegetativnih lastnosti poganjkov v primerjavi z BA.

Okrajšave: BA: 6-benzil adenin.
NAA: α-naftalen ocetna kislina.
MS: Murashige in Skoog bazalno gojišče (Murashige in Skoog, 1962)

Ključne besede: *in vitro*, benzil adenin, zalistni brsti, kinetin, naftalen ocetna kislina, razmnoževanje s stebelnimi izsečki

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1 INTRODUCTION

The pineapple plant (*Ananas comosus* (L.) Merr.) belongs to bromeliad family (Bromeliaceae), which contains 50 genera and about 2500 known species (Duval *et al*., 2003). It is a perennial, monocotyledonous plant having a terminal inflorescence and a terminal multiple fruit (OGTR, 2003). South America is the original home of this plant, which has spread from it through immigration to Central and North America (Bertoni, 1919). Crown, slips, suckers and stem sections have all been commonly utilized for vegetative multiplication of the pineapple (OGTR, 2003). Many studies showed million of pineapple plants can be produced by tissue culture of the crown or shoot tip per year. They reported the rate of multiplication and total number of plantlets produced using plant growth regulators (Sripaoraya *et al*., 2003; Be and Debergh, 2006; Hamad and Taha, 2008). Khan *et al*. (2004) found that the terminal buds of pineapple plant cultured on full and half strength MS medium supplemented with 5.0 mg.l^{-1} BA gave better from other studied concentrations in the number and length of shoots per explant. Abul-Soad *et al*. (2006) got similar results when culturing *in vitro* axillary buds of pineapple plant on full strength MS medium supplemented with 1.0 mg l^{-1} BA and kinetin. Also, that the terminal buds cultured on MS medium supplemented with BA at 2.0 mg.l^{-1} gave high number and length of shoots (Al-Saif *et al*., 2011). The aim of present study is testing of axillary buds and nodal segments as explants for the propagation of pineapple plant by shoot multiplication technique.

2 MATERIALS AND METHODS

2.1 The first experiments: Effect of different concentrations of BA on shoots formation from axillary buds culture

The fresh pineapple fruits with bright green crowns were used for the experiment. Then The green crowns anatomized for the purpose of obtaining the axillary buds at Plant Tissue Culture Laboratory, College of Agriculture, University of Basrah. As the axillary bud excises with the part of leaf base. These explants were put in antioxidant solution consisting of citric acid (150 mg l^{-1}) and ascorbic acid (100 mg l^{-1}) in the refrigerator for 24 hours to avoid phenolic compounds exudation during explants culturing. These explants were then rinsed with sterile distilled water for 3 times and surface sterilized with 20% commercial chlorax solution containing 1.05% sodium hypochlorite, and a drop of tween 20 for 15 minutes. The explants were rinsed in sterile distilled water for 3 times. Immediately after the sterilization process, the explants cultured on full strength of MS medium (Murashige and Skoog, 1962) supplemented with the organic components referred in table 1. The cultures were grown in a growth room at 27±1 °C and 16 hours light and 8 hours dark. The axillary buds did not grow after 4-5 months of culture. There have been re-cultured in the same medium used in first culture.

Table 1: The chemical composition additives to MS medium used for axillary buds culture.

Seq.	Chemical material	Quantity (mg l^{-1})
1	Sucrose	30000
2	Poly vinyl pyrolodine	2000
3	Thiamin-HCl	2
4	Biotin	2
5	Glycine	2
6	Adenine sulphate	40
7	Agar	6000
8	Naphthalene acetic acid	0.2
9	Benzyl adenine	1.0, 1.5, 2.0, 2.5 or 3.0
10	Activated charcoal	500

2.2 The second experiments: Effect of cytokinin type and concentration on shoots formation from nodal segment culture

In this experiment used the same of the last medium components with two cytokinins (benzyl adenine and kinetin) tested at two concentrations (0.5 and 1.0 mg l^{-1}) for each of them. The nodal segments used were taken from shoot produced from indirect organogenesis of callus induced in the first experiment. They were 1.0 cm length and contain 2-3 nods with removal of shoot tips (Figure1 D). The data records after eight months from culture.

The characteristics studied:

1. Number of shoot/explant
2. Leaf length (cm)
3. Number of leaves/shoot
4. Leaf area (cm^2).

Statistical analysis:

Data were statistically analyzed in a completely randomized design with five replicates. Mean values were compared using revised LSD at 5% (Snedecor and Cochran, 1986).

3 RESULTS AND DISCUSSION

3.1 Effect of different concentrations of BA on shoots formation from axillary buds culture

Axillary buds did not grow during 4-5 months of culturing in the MS medium prepared for the shoot multiplication. There growth has been slow because of the buds dormancy. This result is in accordance with Roy *et al*. (2000), when they were propagated *in vitro* pineapple plant. But the axillary buds grew after re-cultured on the same MS medium after two months (Table 2).

Results in the table 2 showed that axillary buds were vegetative growth without callus induction after two months from culturing on full strength MS medium supplemented with 1.0 mg l^{-1} BA. The other MS mediums with different concentrations of BA led to stopped growth of axillary buds, but the growth of callus occurred. Amount of callus increased with increasing concentration of BA added to MS medium except for concentration of BA at 3.0 mg l^{-1} which led to browning of axillary buds and death with very small amount of callus (Table 2). The results showed that the concentration of BA at 2.5 mg l^{-1} added to MS medium are given a very large amount of callus compared to other studied concentrations of BA. The callus induction without shoots formation was related to the explant, type and concentration of cytokinin used in the experiment that stimulated cell division and callus formation without producing lateral shoots (Chanana and Gill, 2008; Al-Taha *et al*., 2012).

The results of the present study are agreement with the results of other studies related to the culturing of *in vitro* pineapple buds taken from crowns of fruits in the full strength MS medium supplemented with different concentrations of BA (Akbar *et al*., 2003; Amin *et al*., 2005; Al-Taha *et al*., 2012). That the reason to increase the amount of callus culturing from MS medium supplemented with 2.5 mg l^{-1} BA due to be supra-optimal for cell division stimulation and callus formation (Al-Taha, 2008).

The callus grown in the MS medium supplemented with 1.0 mg l^{-1} BA and 0.2 mg l^{-1} NAA induced indirect shoots growth after eight weeks from culture(Figure 1 A and B). The nodal segments (explants) taken from shoots obtained by organogenesis were used in the second experiment (Figure 1 C). Organogenesis was induced by the components of MS medium that led cells dedifferentiation of callus that had grown and developed into vegetative shoots (Thorpe, 1978). These results are in accordance with results of other studies on indirect organogenesis from callus of pineapple plant cultured on MS medium supplemented with cytokinin and auxin (Akbar *et al*., 2003; Khan *et al*., 2004; Al-Taha *et al*., 2012).

Table 2: Effect of different concentrations of BA added to MS medium prepared for growth of axillary buds of pineapple plant.

Conc. of BA (mg l^{-1})	Notes	Amount of callus
1.0	Axillary bud grew vegetative and it produced small shoot.	-
1.5	Axillary bud grew slightly with be callus greenish color.	*
2.0	Axillary bud did not grow and be callus grainy whitish green.	**
2.5	Axillary bud did not grow and be callus grainy whitish green.	***
3.0	Axillary bud died and brown discoloration and callus be very small amount.	*

(-): Mean not grow callus.
(*): Means the growth of a small amount of callus.
(**): Means the growth of a large amount of callus.
(***): Means the growth of a very large amount of callus.

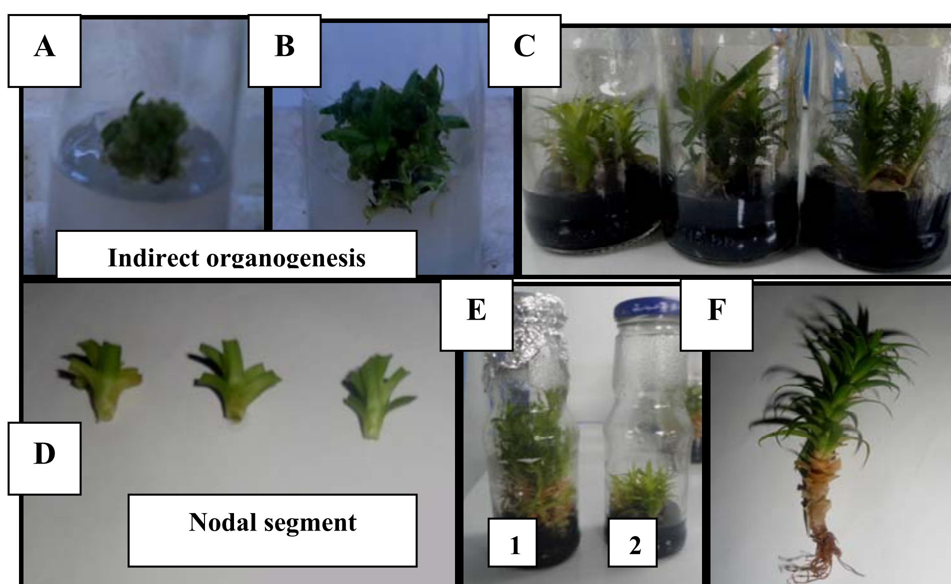

Figure 1: Effect of source of explant and cytokinin on formation shoots of *in vitro* propagation of pineapple plant (*Ananas comosus* 'Queen').

A, B and C: The stages of indirect shoots from culturing of callus of axillary buds on MS medium supplemented with (1.0 mg l^{-1} BA and 0.2 mg l^{-1} NAA) after two months.
D: Nodal segments (2-3 nodes) used in the second experiment of shoots multiplication.
E: Formation shoots cultured on MS medium supplemented with 1.0 mg l^{-1} kinetin (1) 0.5 mg l^{-1} BA (2).
F: Shoot produced from shoots multiplication of nodal segment cultured on MS medium supplemented with 1.0 mg l^{-1} kinetin.

3.2 Effect of cytokinin type and concentration on shoots formation from nodal segments culture

Table 3 showed the effect of BA and kinetin at (0.5 or 1.0 mg l^{-1}) added to full strength MS on vegetative characteristics of induction shoots from nodal segments after eight weeks from culturing. Number of multiplication shoots/explant, leaf length (cm), number of leaves/shoot and leaf area (cm^2) were increased with increasing cytokinin (BA or kinetin) added to MS medium. This result

is in accordance with results of other studies on effect of cytokinins on shoots multiplication of *in vitro* propagation of pineapple plant (Kiss *et al*., 1995; Bhatia and Ashwath, 2002; Abul-Soad *et al*., 2006; Be and Debergh, 2006).

The results of Table 3 showed that MS medium supplemented with kinetin at 1.0 mg l^{-1} was a significant superior than other treatments of MS media. It gave highest rate in vegetative characteristics of formation shoots (18.60 shoots/explant, 5.38 cm of leaf length (Figure 1 F), 10.60 leaves/shoot and 3.64 cm^2 of leaf area) except leaf area which did not significant different with leaf area of MS medium supplemented with 1.0 mg l^{-1} BA. While the MS medium supplemented with 0.5 mg l^{-1} BA led to a significant decline in all vegetative characteristics reached (7.00 shoots/explant, 2.72 cm, 4.80 leaves/shoot and 1.74 cm^2), respectively when compared with other treatments of MS media (Figure 1 E1 and 2).

The same table showed that MS medium supplemented with kinetin at (0.5 or 1.0 mg l^{-1}) was a significant superior on BA in all vegetative characteristics of formation shoots except the treatment of kinetin at 1.0 mg l^{-1} which was not significantly different from 1.0 mg l^{-1} BA in leaf area. The reason for this is that kinetin was probably more effective than BA in stimulation of adventitious shoots production.

Table 3: Effect of cytokinin type and concentration on vegetative characteristics of formation shoots from nodal segments of pineapple plant culture in full strength MS medium after eight weeks.

Treatment (mg l^{-1})		No. of shoots/explant	Leaf length (cm)	No. of leaves/shoot	Leaf area (cm^2)
BA	0.5	7.00	2.72	4.80	1.74
	1.0	13.60	4.12	9.40	3.42
Kinetin	0.5	10.60	3.80	7.60	2.94
	1.0	18.60	5.38	10.60	3.64
Revised-LSD (5%)		1.912	0.382	1.175	0.355

4 CONCLUSIONS

The source of explant used in tissue culture of pineapple plant has an important role in shoot multiplication. The nodal segments as explants gave adventitious shoots while axillary buds did not develop into the shoots but grew callus. As we can deduce from the results of this study kinetin was more effective than BA in shoots multiplication.

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