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Effects of some plant growth regulators and different culture media on *in vitro* shoot multiplication of Photinia (*Photinia x fraseri*)

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Abstract

The present study was carried out to examine the influence of some plant growth regulators and different culture media on *in vitro* culture of Photinia. Lateral buds were used as explants and surface sterilized by dipping explants in a solution containing 0.1% (w/v) mercury chloride ($HgCl_2$) for 5 min. to determine the most suitable medium and plant growth regulators for shoot multiplication, explants were excised and cultured in different media (Murashige and Skoog (18) (MS); White (30); Gamborg (12) (B5); and Driver and Kuniyuki walnut (11)(DKW) containing various concentrations of Kinetin (Kin) (1, 2 and 3 mgl^{-1}) and Indole-3-butyric acid (IBA) (0.0, 0.2, and 0.4 mgl^{-1}) and their interactions. The best number of shoots per explant (3.2 shoots/ explant) was produced on MS plus 2 mgl^{-1} of Kin and IBA at 0.2 mgl^{-1} and the highest number of leaves per explant (12.20 leaves/ explant) was produced on DKW at 2mgl^{-1} of Kin and IBA at 0.2mgl^{-1}, this increases was significantly which compared to all treatments except this treatment containing (DKW + Kin 1, 2 mgl^{-1}+ 0.4mgl^{-1} IBA). DKW medium was recorded the highest average of shoots length/ explant when medium containing both Kin at 2 mgl^{-1} with 0.2 mgl^{-1} of IBA as compared with others combinations.

Keywords: *In vitro* multiplication, Photinia (*Photinia x fraseri*), Plant growth regulators, MS, White, B5 and DKW medium.

culture techniques in which a large number of shoots was produced when explants were cultured in nutrient media supplemented with different plant growth regulators. The selection of a culture medium is an essential step in any plant tissue culture; the culture medium must supply all the essential elements and nutrients necessary for the growth of plants *in vitro*. Plant tissue culture medium should contain some or all of the following components: macronutrients, micronutrients, vitamins, amino acids, nitrogen complements, source (s) of carbon, undefined organic complements, growth regulators and solidifying agents. The best balance of minerals in the basal medium is critical in promoting healthy plant growth *in vitro* and in control of growth disorders which are oftentimes related to mineral deficiency or toxicity Ashrafi *et al.* (4). Several medium kinds are commonly used for the most of all cell and tissue culture such as

Introduction

Photinia (*Photinia x fraseri*) is a member of *Rosaceae* family, which is a popular evergreen, woody shrub, with glossy green leaves, young red shoots and fascinating white flowers. It is cultured as a shrub or as a fast-growing, evergreen hedge. Akdemir *et al*. (2) reported there is an increasing demand of Photinia production, however, vegetative propagation techniques is not practical to be fast and clonal propagation method. Wu (31) stated a micropropagation techniques that can proliferation of a large number of shoots from apical meristems of this species.

In vitro culture is one of the key tools of plant biotechnology that exploits the tot potency nature of plant cells, an idea proposed by Haberlandt (14) and unequivocally explained for the first time by Steward *et al*. (27). The multiplication stage was a very important stage in plant propagation through *in vitro*

study lateral buds of about 1cm as explants were used, these explants were excised and washed with tap water for an hour with liquid soap every 10 min, to remove dirt's. For surface sterilization explants were dipped in a solution containing 0.1% (w/v) mercury chloride ($HgCl_2$) for 5 min. After surface sterilization, explants were rinsed four times in sterile distilled water for 5 min to remove the harmful effects of the sterilant. Explants were cultured on Murashige and Skoog (18) Medium containing Benzyl adenine (BA) (2 mgl^{-1}) to obtain sterile explants and after one week sterilize explants transfer to different mediums. To determine the most suitable medium and concentration of Kinetin, Indole-3-butyric acid and their combinations on shoot multiplication, explants were excised and cultured on different mediums (Murashige and Skoog (18) (MS); White (30); Gamborg (12) (B5); and Driver and Kuniyuki walnut (11) (DKW) Nitsch (19); Murashige and Skoog (18) (MS); White (30); Limsmaier and Skooge (15) (LS); Gamborg (12) (B5); Woody plant medium (WPM) Lloyd and McCown (16), Quoirin and Lepoivre (QL)(23) and others. Murashige and Skoog medium (18) is usually used to establish and keep most types of plant tissue cultures(26 and 29). Plant growth regulators that regulate growth and morphogenesis and catalyze cell division and they were shown to affect many other physiological and developmental processes. This experiment has been performed in order to evaluate the suitable plant growth regulator and different culture media on *in vitro* shoot multiplication of Photinia.

Materials and Methods

The present study was carried out in the plant tissue culture laboratory, Faculty of Agriculture, University of Dohuk, Kurdistan Region, Iraq, during the year, 2014. In these

explant (2.17 shoots/ explant) was found on white medium compared with MS and B_5 media, and (2.03 and 2.01 shoots/ explant) was observed on medium containing 2mgl^{-1} Kin and 0.2 mgl^{-1} IBA alone, respectively. While, the combination between two factors different culture media with different concentrations of Kin, different culture media with different concentration of IBA and interaction between Kin +IBA on number of shoots. The maximum number of shoots (2.4, 2,26 and 2.25 shoots/ explant) was observed on MS medium having 3mgl^{-1} Kin and white medium having 2mgl^{-1}Kin, white media containing (0.2 or 0.4) mgl^{-1} IBA and medium containing 2mgl^{-1} Kin+ 0.2mgl^{-1}IBA respectively.

The combination between different media and concentrations of Kin and IBA, was recorded the best number of shoots per explant (3.2 shoots/ explant) was produced on MS at 2 mgl^{-1} of Kin and IBA at 0.2 media containing various concentrations of Kinetin (Kin) at 1, 2 and 3 mgl^{-1} and Indole-3-butyric acid (IBA) at 0.0, 0.2, and 0.4 mgl^{-1}. One explant was cultured in each jar and 5 replicates were used for each treatment. Cultures were grown in the growth room for eight weeks, and then observations on the number of shoots, average of shoots length, number of nodes and leaves number were recorded. The experiments were arranged according to Complete Randomized Design (CRD). Data were analyzed and means were compared with each other's using Duncan's multiple range test at 0.05 level. All statistical analysis was performed using the computerized program of SAS (24).

Results

1. Number of shoots/ explant

Table (1) show the effect of different media, Kin and IBA alone on number of shoots, the highest number of shoots per

mgl^{-1} as shown in figure (1). While the minimum number of shoots (1 shoots/ explant) was produced on B_5 medium containing $1mgl^{-1}$ Kin+ $0.4mgl^{-1}$ IBA.

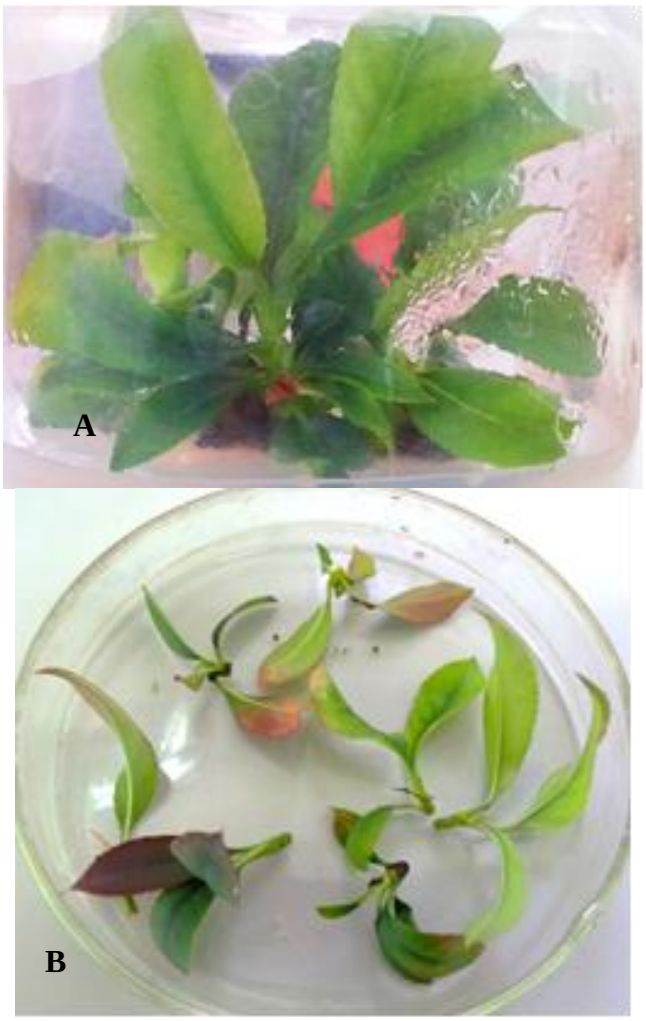

Figure (1): Explant cultured on MS medium supplemented with Kin at 2 mgl^{-1} and IBA at 0.2 mgl^{-1}. A. Explant cultured on MS medium after eight weeks of culture before separation. B. Explant cultured on MS medium after eight weeks of culture after separation.

Table (1): Effect of different culture media, Kin and IBA and their combination on shoots number/ explant, of Photinia after 4 weeks.

Media	Kin (mgl^{-1})	IBA (mgl^{-1})			Media x Kin	Means of Media
		0	0.2	0.4		
MS	1	1.0 d	1.0 d	1.0 d	1.0 d	1.68 b
	2	1.2 cd	1.4 cd	2.4 a-c	1.66 bc	
	3	1.8 b-d	3.2 a	2.2 a-d	2.4 a	
White	1	1.4 cd	2.0 b-d	2.2 a-d	1.86 a-c	2.17 a
	2	2.4 a-c	2.4 a-c	2.4 a-c	2.4 a	
	3	2.2 a-d	2.4 a-c	2.2 a-d	2.26 ab	
B5	1	2.0 b-d	2.0 b-d	1.0 d	1.66 bc	1.68 b
	2	1.6 b-d	2.4 a-c	1.8 b-d	1.93 a-c	
	3	1.2 cd	1.2 cd	2.0 b-d	1.46 cd	

DKW	1	1.4 cd	2.2 a-d	2.4 a-c	2.0 a-c	1.95 ab
	2	1.8 b-d	2.8 ab	1.8 b-d	2.13 ab	
	3	1.6 b-d	1.2 cd	2.4 a-c	1.73 bc	
Media x IBA	MS	1.33 c	1.86 a-c	1.86 a-c	Mean of Kin	
	White	2.0 ab	2.26 a	2.26 a		
	B5	1.6 bc	1.86 a-c	1.6 bc		
	DKW	1.6 bc	2.06 ab	2.2 ab		
Kin x IBA (mgl^{-1})	1	1.45 c	1.8 a-c	1.65 bc	1.63 b	
	2	1.75 a-c	2.25 a	2.1 ab	2.03 a	
	3	1.7 a-c	2.0 ab	2.2 ab	1.96 a	
means of IBA		1.63 b	2.01 a	1.98 a		

*Means followed by the same letter for each factor and interaction do not differ significantly from each other's according to Duncan's Multiple Range Test at 5% level.

+IBA on number of shoots. The maximum number of shoots (2.84 cm) was observed on DKW medium having 2mgl^{-1} Kin, DKW medium containing 0.4mgl^{-1} IBA. While the maximum average of shoots

length/ explant was obtained when explants were planted on DKW medium plus0.4mgl^{-1} IBA. However the medium containing 2mgl^{-1} Kin+ 0.2mgl^{-1}IBA produced an average of shoots length/ explant (2.32 cm), and it was non significantly increases as compared with all treatments only treatment which containing 1mgl^{-1}Kin + (0, 0.2)mgl^{-1} IBA and treatment which supplemented 3mgl^{-1} Kin+ 0.4mgl-1 IBA .

The consequence of the three combination between different media and different concentrations of Kin and IBA, on the average of shoots length/ explant (3.22 cm) was produced

2. Shoots length

Table (2) showed the effect of different culture media, Kin and IBA alone on shoots length/ explant, the longest shoots per explant (2.67cm) was found on DKW medium and it was significantly increases as compared with all media, and (2.06 and 2.08cm) was observed on medium containing 3mgl^{-1} Kin and 0.4 mgl^{-1} IBA alone, respectively, the increases were significantly compared with medium having 1mgl^{-1} Kin and 0 IBA.

Results appeared that when explants were planted in media with interactions between different mediums Kin and IBA on the average of shoots length/ explant, the combination between different culture media with different concentration of Kin, different culture media with different concentration of IBA and interaction between Kin

Table (2): Effect of different mediums, Kin, IBA and their combinations on average of shoots length/ explant of Photinia after 4 weeks.

Media	Kin (mgl^{-1})	IBA (mgl^{-1})			Media x Kin	Means of Media
		0	0.2	0.4		
MS	1	1.22 jk	1.5 h-k	2.3 b-g	1.67 f-h	2.0 b
	2	2.56 b-e	2.4 b-g	2.02 e-h	2.32 cd	
	3	2.12 d-h	2.14 c-h	1.76 f-k	2.0 d-f	
White	1	1.12 k	1.16 k	1.2 jk	1.16 i	1.4 c
	2	1.2 jk	1.22 jk	1.9 e-i	1.44 hi	
	3	1.38 i-k	2.04 e-i	1.4 i-k	1.6 gh	
B5	1	1.86 f-j	2.36 b-g	2.14 c-h	2.12 de	2.06 b
	2	2.3 b-g	2.44 b-f	1.72 g-k	2.15 c-e	
	3	1.86	1.9	2.0	1.92 e-g	

		f-j	e-i	e-i		
DKW	1	2.02 e-i	2.44 b-f	2.96 ab	2.47 bc	2.67 a
	2	2.56 b-e	3.22 a	2.74 a-d	2.84 a	
	3	2.74 a-d	2.56 b-e	2.82 a-c	2.7 ab	
Media x IBA	MS	1.96 d	2.01 d	2.02 d	Means of Kin	
	White	1.23 e	1.47 e	1.5 e		
	B5	2.0 d	2.23 cd	1.95 d		
	DKW	2.44 bc	2.74 ab	2.84 a		
Kin x IBA (mgl^{-1})	1	1.55 c	1.86 b	2.15 ab	1.85 b	
	2	2.15 ab	2.32 a	2.09 ab	2.19 a	
	3	2.02 ab	2.16 ab	1.99 b	2.06 a	
means of IBA		1.91 b	2.11 a	2.08 a		

*Means followed by the same letter for each factor and interaction do not differ significantly from each other's according to Duncan's Multiple Range Test at 5% level.

minimum number of shoots (1.12 cm) was produced on white medium containing 1mgl^{-1} Kin and(0) IBA.

on DKW at 2mgl^{-1} of Kin and IBA at 0.2mgl^{-1} the increases was significantly compared with the majority of treatments as appeared in figure (2). While the

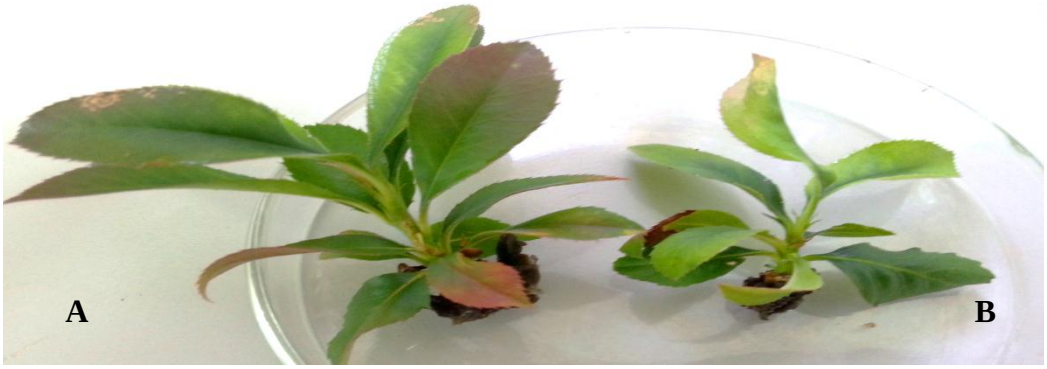

Figure (2): Effect of different mediums, Kin, IBA and their interactions on average of shoots length/ explant. A. Explant planed on DKW medium contains 2 mgl^{-1} of Kin and 0.2 mgl^{-1} of IBA. B. Explant planed on MS medium contains Kin at 2 mgl^{-1}.

was DKW as indicated in Table (3) and (5.11 and 4.78 nodes/ explant) was observed on medium containing 2mgl^{-1} Kin and 0.4 mgl^{-1} IBA alone, respectively, the increases was significantly compared with medium having 1,3 mgl^{-1} Kin and 0 IBA.

3. Nodes number/ shoot

Table (3) showed that the maximum number of nodes/ explant (6.42 nodes/ explant) was found on DKW medium. During the culture period, the number of nodes had significant increased when culture medium

Table (3): Effect of different media, Kin, IBA and their combinations on nodes number/ shoot of Photinia after 4 weeks.

Media	Kin (mgl^{-1})	IBA (mgl^{-1})			Media x Kin	Means of Media
		0	0.2	0.4		
MS	1	3.2 g-k	3.2 g-k	4.4 d-i	3.6 de	4.55 b
	2	4.8 c-h	7.6 a	5.0 c-g	5.8 ab	
	3	4.4 d-i	4.8 c-h	3.6 f-k	4.26 cd	
White	1	2.0 k	3.0 h-k	2.4 jk	2.46 f	3.11 d
	2	2.4 jk	2.6 i-k	4.8 c-h	3.26 ef	
	3	3.2 g-k	4.2 e-j	3.4 f-k	3.6 de	
B5	1	3.4 f-k	3.6 f-k	4.0 e-i	3.66 de	3.95 c
	2	5.8 a-e	4.8 c-h	4.8 c-h	5.13 bc	
	3	2.6 i-k	2.4 jk	4.2 e-j	3.06 ef	

DKW	1	5.6 b-e	6.6 a-c	6.6 a-c	6.26 a	6.42 a
	2	5.2 b-e	7.0 ab	6.6a -c	6.26 a	
	3	6.2 a-d	6.4 a-c	7.6 a	6.73 a	
Media x IBA	MS	4.13 de	5.2 bc	4.33 cd	Means of Kin	
	White	2.53 f	3.26 ef	3.53 de		
	B5	3.93 de	3.6 de	4.33 cd		
	DKW	5.66 b	6.66 a	6.93 a		
Kin x IBA (mgl^{-1})	1	3.55 d	4.1 cd	4.35 cd	4.0 b	
	2	4.55 c	5.5 a	5.3 ab	5.11 a	
	3	4.1 cd	4.45 c	4.7 a-c	4.41 b	
means of IBA		4.06 b	4.68 a	4.78 a		

*Means followed by the same letter for each factor and interaction do not differ significantly from each other's according to Duncan's Multiple Range Test at 5% level.

medium supplemented with $2mgl^{-1}$ of Kin and IBA at $0.2mgl^{-1}$, the increases was significantly compared with the majority of treatments. While the minimum number of nodes/ explant (2.00) was produced on white medium containing $1mgl^{-1}$ Kin without IBA

4. Number of leaves/ shoot

Table (4) shows effect of different culture media, Kin and IBA alone on number of leaves, the highest number of leaves per explant (10.04 leaves / explant) was found on DKW medium and it was significantly increases as compared with all media, and (7.77and 7.63 leaves / explant) was observed on medium containing $2mgl^{-1}$ Kin and 0.4 mgl^{-1} IBA alone, respectively, the increases significantly.

The same Table illustrated effect of different media Kin, IBA and their interactions on number of

Results appeared that when explants were cultured on media with interactions between different levels of Kin and IBA has significant effect on number of nodes/ explant. The maximum number of shoots (6.73 and 6.93 nodes/ explant) was observed on DKW medium having $3mgl^{-1}$ Kin and the maximum number of nodes/ explant was obtained when explants were planted on DKW medium plus $0.4mgl^{-1}$ IBA. However the medium containing $2mgl^{-1}$ Kin+ $0.2mgl^{-1}$IBA produced an average of shoots length/ explant (5.5 nodes/ explant), and it was non

significant increases as compared with other treatments.

Results of three combination between different media and different concentrations of Kin and IBA, on number of nodes/ explant (7.6 nodes/ explant) was produced on DKW at $3mgl^{-1}$ of Kin and IBA at $0.4mgl^{-1}$ and MS

Table (4): Effect of different media, Kin, IBA and their combinations on leaves number/ shoot of Photinia after 4 weeks.

Media	Kin (mgl^{-1})	IBA (mgl^{-1})			Media x Kin	Means of Media
		0	0.2	0.4		
MS	1	4.8 j-m	5.2 i-m	7.0 d-k	5.66 c	6.96 b
	2	8.4 c-h	7.6 c-i	7.2 c-j	7.73 b	
	3	7.6 c-i	7.8 c-i	7.0 d-k	7.46 b	
White	1	3.00 m	5.2 i-m	4.0 lm	4.06 d	5.04 c
	2	4.0 lm	4.0 lm	6.8 e-k	4.93 cd	
	3	5.4 i-m	6.4 g-l	6.6 f-l	6.13 c	
B5	1	6.4g -l	5.8 h-l	5.8 h-l	6.0 c	6.28 b
	2	8.2 c-h	8.0 c-i	7.2 c-j	7.8 b	
	3	4.0 lm	4.4 k-m	6.8 e-k	5.06 cd	

DKW	1	8.8 c-g	9.4 b-e	11.8 ab	10.0 a	10.04 a
	2	7.8 c-i	12.2 0 a	11.80 ab	10.60 a	
	3	9.8 a-c	9.2 c-f	9.6 b-d	9.53 a	
Media x IBA	MS	6.93 c	6.86 c	7.06 c	Means of Kin	
	White	4.13 e	5.2 de	5.8 cd		
	B5	6.2 cd	6.06 cd	6.6 cd		
	DKW	8.8 b	10.2 6 a	11.07 a		
Kin x IBA (mgl^{-1})	1	5.75 d	6.4 cd	7.15 ac	6.43 b	
	2	7.1 a-c	7.95 ab	8.25 a	7.77 a	
	3	6.7 b-d	6.95 b-d	7.5 a-c	7.05 b	
means of IBA		6.52 b	7.10 ab	7.63 a		

*Means followed by the same letter for each factor and interaction do not differ significantly from each other according to Duncan's Multiple Range Test at 5% level.

Figure (2): Effect of different mediums, Kin, IBA and their interactions on number of leaves of Photinia explant. A. Explant cultured on DKW medium supplemented with Kin at 2 mgl^{-1} and IBA at 0.2 mgl^{-1}. B. Explant cultured on B5 medium supplemented with Kin at 2 mgl^{-1}. C. Explant cultured on WHITE medium contains 2 mgl^{-1} Kin.

significantly increases as compared some treatments.

The combination between different media and different concentrations of Kin and IBA, the maximum number of leaves per explant (12.20 leaves/ explant) was produced on DKW at 2mgl^{-1} of Kin and IBA at 0.2mgl^{-1} the increases was significantly compared all treatments without this treatment containing (DKW + 1,2 mgl^{-1}+ 0.4mgl^{-1} IBA). While the minimum number of shoots (3 leaves/ explant, the combination between different culture media with different concentration of Kin, different culture media with different concentration of IBA and interaction between Kin +IBA on number of shoots. The maximum number of shoots (10.60, 11.07 and 8.25 leaves/ explant) was observed on DKW medium having 3mgl^{-1} Kin, DKW medium containing 0.4mgl^{-1} IBA and medium containing 2mgl^{-1} Kin+ 0.4mgl^{-1}IBA respectably, and it was

(3) explained the positive effect of Kin in increasing the number of shoots and their length that could be due to the role of the Kin in obstruction the destruction of the protein and chlorophyll, also he also explained that Kin promoted photosynthesis enzymes that reflected in increasing the cell size, promoting cell division and the morphogenesis processes

As appeared from the current results, the number of shoots, shoots length, number of nodes and leaves number per explants increased by addition of IBA as compared with IBA-free medium, this might be due to auxins promotes cell division, cell enlargement (22).

Whereas the interaction between different concentrations of Kin and IBA, there is a significant increase in number of shoots, shoots length, number of nodes and leaves number / explants as shown in the tables. This may be due to the role of auxin and cytockinin in the cell division leaves/ explant) was produced on white medium containing 1mgl^{-1} Kin without IBA as cleared in figure (3).

Discussion

Plantlet morphgenesis could be explained by the effect of plant growth regulators as well as the components of the basal medium. Most of the results in the tables showed that the number of shoots and leaves, shoots length and number of nodes per explants increased by using Kin at concentration of 2.0mgl^{-1} as compared with most other treatments. The positive effect of Kin on multiplication stage might be due to the great role of cytokinins in lateral buds from the dominance by promoting the formation of xylem tissues of buds which will facilitate the transformation of water and nutrients leading to lateral bud growth (17). Cline (7) reported that cytokinins considered as important factors in controlling and breaking the dormancy and apical dominance

suggested that the explants were successfully established in DKW medium supplemented with 0.5 mgl^{-1} of BAP and Tang *et al.* (28) who used DKW medium for tissue culture of sour cherry (*Prunus cerasus* L.).

While the best medium for number of shoots/ explant was appeared on MS medium which produce 3.2 shoots/ explant at 2 mgl^{-1} of Kin and at 0.2 mgl^{-1} of IBA, followed DKW which produced 2.8 shoots/ explant as appeared in the results, mainly may due to the salt mixtures in MS that provide a balance and suitable nutrients for *in vitro* growth of most plant species (5), as the medium contain high macro and micro nutrients (6) stated that the medium varied at most in nitrogen content, higher in MS (60mM) while DKW (44mM). Similar results were observed by Abdi (1) who suggested that when explants cultured on MS, B5 and SH medium, the highest shoot proliferation response observed successfully by using MS and as a result rapid differentiation of shoots and leaves formation (3). Also George *et al*. (13) illustrated that auxins at most in interaction with cytokinins promote the growth of organs, and also regulate the direction of morphogenesis.

In addition to the effect of plant growth regulators, culture medium also effect on explants multiplication as clear from the above results the number of leaves, average shoots length and number of nodes/ explants increased when explants planted on KDW medium as compared with MS, B5 and White medium, could be due to the DKW medium, contain high level of many nutrients, like magnesium, manganese, zinc, and nickel(8). Shoot multiplication rate also different in dissimilar species and was specific to the culture medium (20, 25 and 9) explained that the degree of growth and differentiation varied a lot with the medium constitution. Similar results have been recorded by Dejampour *et al.* (10) who

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medium containing 4 mg l^{-1} BA. Also Parris *et al*. (20) mentioned that the maximum number of shoots developed on full strength MS while DKW-simulated shoot elongation.

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تأثير بعض منظمات النمو النباتية و الاوساط الغذائية على تضاعف نبات الفيتونيا (*Photinia x fraseri*) خارج الجسم الحي

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المستخلص

أجريت هذه الدراسة لدراسة تأثير بعض منظمات النمو النباتية و اوساط غذائية مختلفة على زراعة نبات الفيتونيا داخل المختبر. استخدمت البراعم الجانبية كأجزاء نباتية حيث عقمت بغمر الاجزاء النباتية في محلول حاوي على 0.1% كلوريد الزئبق ($HgCl_2$) لمدة 5 دقائق . لتقدير افضل وسط و افضل منظمات النمو النباتية لتضاعف النبات. اخذت الاجزاء النباتية و زرعت على اوساط غذائية مختلفة (Murashige and Skoog (1962) (MS); White (1963); Gamborg (1968) (B5); Driver and Kuniyuki walnut (DKW)) التي تحتوي على تركيزات مختلفة من Kinetin (Kin) (1، 2 و3 ملغم. لتر $^{-1}$) و اندول بيوتريك اسيد (IBA) (0.0، 0.2 و 0.4 ملغم. لتر$^{-1}$) و تداخلاتهما. افضل عدد الافرع لجزء النباتي (3.2 فرع/ جزء نباتي) أنتجت عند زراعة الجزء النباتي على وسط MS 2ملغم لتر$^{-1}$ Kin و IBA عند 0.2 ملغم لتر$^{-1}$ و اعلى عدد اوراق لجزء النباتي (12.20 اوراق/ جزء نباتي) أنتجت عند زراعة الجزء النباتي على وسط DKW 2 ملغم. لتر$^{-1}$ Kin و IBA عند 0.2 ملغم. لتر$^{-1}$هذه الزيادة كانت زيادة معنوية مقارنة مع جميع المعاملات الاخرى ما عدا المعاملة الحاوية (1 Kin+DKW، 2 ملغم. لتر$^{-1}$ + 0.4 ملغم. لتر$^{-1}$ IBA). وسط DKW سجل اعلى معدل طول الافرع لجزء النباتي عند وسط حاوي على Kin 2 ملغم. لتر$^{-1}$ مع 0.2 ملغم. لتر$^{-1}$ IBA مقارنة مع تداخلات الاخرى.

الكلمات المفتاحية: التضاعف خارج الجسم الحي، نبات الفيتونيا، منظمات النمو النباتية، الاوساط الغذائية (Ms ، White ، B5 و DKW)