

IN VITRO MULTIPLICATION OF PHOTINIA (*PHOTINIA X FRASERI*) USING DIFFERENT CULTURE MEDIA

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ABSTRACT:

A very high rate of multiple shoots was obtained from nodal explants of *Photinia x fraseri* on MS medium supplemented with a various concentration of Kin alone (1, 2, and 3) mg l^{-1} alone and combination of Kin at (1, 2, and 3) mg l^{-1} with IBA or IAA at (0.2 and 0.4) mg l^{-1} . The results show that the number of shoots/ explant was significantly affected the levels of Kin alone and combination with IBA or IAA. The number of shoots (6.00 shoot/ explant) when Kin added to the medium at 3mg l^{-1} in which it was significantly different from other Kin concentrations. While the effect of different medium with the fixed and best concentration of Kin+ IBA and Kin+ IAA on the average shoot number and leaves number /explant of *Photinia* explant (node segment and shoot tips) The highest number of shoots (5.90) was recorded in node segment when cultured on WPM containing Kin+ IBA and it was increased significantly compared with all treatments from shoot tip. However, the maximum number of leaves (20.10) was recorded on WPM medium supplemented with Kin+ IBA and it was increased significantly compared with all treatments which containing different media plus Kin at $2\text{mg l}^{-1} + 0.4\text{mg l}^{-1}$ IBA and Kin at $1\text{mg l}^{-1} + 0.4\text{mg l}^{-1}$ IAA. Shootlet form the combination of from *photinia* cultured on $\frac{1}{4}$ MS medium improved 0.6 mg l^{-1} NAA concentration produced the highest number of roots/shootlet (6.5 roots/shootlet) compared with all treatments contains $\frac{1}{2}$ MS medium added IAA, IBA and NAA on all concentration. While, the shootlets from *photinia* cultured on $\frac{1}{4}$ MS medium and supplemented with 0.6mg l^{-1} NAA formed an average (5.29 cm) compared to all treatments containing $\frac{1}{2}$ MS medium. Such plantlets were successfully transferred to soil after hardening with a high rate of survival.

Keyword: *Photinia x fraseri*, tissue culture media and plant growth regulators.

INTRODUCTION:

Fraser *Photinia* belongs to Rosacea family it is a general evergreen shrubs with glossy green leaves, white flowers and young red shoots. *Photinia* is a genus of about 40-60 species of small trees and large shrub. They are restricted to warm temperature in Asia, from the Himalaya east to Japan and to India and Thailand (James, 1992). Fraser *Photinia* is an important woody landscape plant used for hedging and screening in the USA (Dirr, 1983 and Dirr and Heuser, 1987). Red tip is used to create majestic tall hedges. Red tip hedges can be left unprimed for "natural" look. They retain foliage to the ground and never become leggy.

The traditional propagation method of *photinia* is by rooting the apical cutting with great concentrations of plant growth regulators (Beeson, 2000). The elongated period required to obtain new plant and the rooting difficulty of cutting are some of the factors that limited the commercial exploitation of this species (Larraburuet *al.*, 2007). Red tip of *photinia* can be propagated using seed exposed to a two-month cold stratification; another method for *photinia* propagation is by tissue culture

technique but the current information on the application of *in vitro* technique for the multiplication of this species is very limited (Kaneet *al.*, 1987; Leifert *et al.*, 1992). The production of woody plants by tissue culture methods is used to facilitate rooting and used to propagation species with rooting difficulties, to solve problems of the seasonal supply associated with the rooting of stem cuttings and to clone disease-resistant specimens and to provide target material for gene transfer (Merkle and Dean, 2000). Terminal and lateral shoots of *photinia* were cut in to node segment of similar size (1cm) and transferred to 300ml flasks with 70ml of medium, supplemented with different concentration of cytokinin according to Leifert *et al.* (1992) and Rafael *et al.* (1997). The shoot length of *photinia* increased significantly when cultured on MS medium containing 2mg l^{-1} BA with average of 23 mm at 28 day of growth and the highest multiplication rate (4.30) shoots were achieved (Larraburnet *al.*, 2007). The medium for multiplication of *Ficus hietawas* $\frac{1}{2}$ MS + 0.5mg l^{-1} BA (Jiang *et al.*, 2004). The highest number of shoots (5 shoot/ explants) of concentration was formed on MS medium containing 4mg l^{-1} BAP compared to (0.69 shoots)

on basal medium. However, shoot regeneration indifferent concentration of Kin was nearly constant (1.18 shoot/ explant). In most concentration of cytokinin, Kin led to produce longer shoots in comparison with BA and as the concentrations of cytokinin increased, the height of shoots decreased (Kharrazi *et al.*, 2011).

Behera and Sahoo (2009) found that the best performance was achieved on MS medium supplemented 2mg l^{-1} BA + 0.5mg l^{-1} IBA on this combination, 92% of the explants produced shoots. Choudhary *et al.* (2011) found that when increased the concentration of BAP in the nutrient media for *Aloe vera* (1BAP and 0.5NAA) mg l^{-1} , the number of shoots were also increased per culture. Photinia were rooted on half strength WPM containing 0.02mg l^{-1} NAA (Lloyd and McCown, 1980). Larrabur *et al.* (2010) used MS medium for photinia shoots rooting supplemented with 10mg l^{-1} IBA. While the best result if root formation was observed on MS medium containing 5mg l^{-1} NAA (Tarique *et al.* 2010). Das (2010) indicated that the maximum percentage of rooting for rose micro-shoots (94%) was noted on medium having half strength of MS medium with 0.25mg l^{-1} IBA within 15 days of cultures.

The aim of study is to identify a suitable explant for shoot induction, a suitable culture media, suitable plant growth regulators and their concentration of shoot multiplication and rooting.

MATERIAL AND METHODS:

The current study was carried out in the Laboratory of plant tissue culture, faculty of Agriculture, university of Dohuk, Kurdistan Region, Iraq, during the period from December 2013 to May 2014. Node segment and shoot tip was used as an explant in this investigation which was taken from an *in vitro* sterile explant and then conducted the following experiments:

For multiplication stage.

1. MS + Kin (1, 2, and 3) mg l^{-1} alone.
2. MS + Kin (1, 2, and 3) mg l^{-1} combination with IBA or IAA at (0.2 and 0.4) mg l^{-1} .
3. The best concentrations from Kin combination with IBA or IAA add to the different media like (WPM, B₅, White and DKW).

All culture media (MS, WPM, B₅, White and DKW) MS (Murashige & Skoog 1962), WPM

(Lloyd and McCown 1980), B₅ (Gam-bor *et al.* 1968), DKW (Driver and Ku-niyuki 1984), supplemented with 3% sucrose, 0.7% agar. After 6-8 weeks, the following data were recorded: Number of shoots, leaves, nodes and Shoot length.

For rooting stage conducted the following experiments:

1. ($\frac{1}{2}$, $\frac{1}{4}$) MS with (0.0, 0.6 and 0.8) mg l^{-1} IBA or IAA or NAA alone.

After 8 weeks, the following data were recorded: Root percentage, Number of root and Root length

Acclimatization stage: After 8 weeks of shoot rooting the plantlets were thoroughly washed with tap water to remove the agar from roots which might be a source of concentration. The plantlets were put in pots containing autoclaved mixture of peat moss and sand in ratio of (1:1/ v: v) placed in sterile boxes covered by polyethylene in order to maintain high relative humidity. The potted plants were placed in incubation room for 30 days. After 4 weeks the plants transferred to the green house.

Data Analysis:

The experiments were arranged according to Complete Randomized Design (CRD) using (5) replication for each treatment. Data were analyzed and means were compared with each other using Duncan's multiple rang test at 0.05 level (Duncan, 1955).

RESULT AND DISCUSSION

Effect of Kin concentration alone and combinations with IBA or IAA on multiplication stage of Photinia explants:

To determine the most suitable concentrations of Kin, IBA or IAA and their combinations on shoot multiplication, the explant were excised and inoculated on MS medium containing different concentrations of Kin with IBA or IAA. As it is clarified in Table (1) the effected of various concentrations of Kin, IBA or IAA and their combinations on shoot number, leaf number, number of node and shoot length of photinia explant after 8 weeks of culture to multiplication stage. It reveals different concentration of Kin, IBA or IAA and their combinations on shoot number. The results show that number of shoots/ explant was significantly affected at levels of Kin alone and

combination with IBA or IAA. The number of shoots (6.00 shoot/ explant) was observed when Kin added to the medium at 3mgL^{-1} which is significantly different from other Kin concentrations.

The combinations between Kin and IBA or IAA increased significantly shoot number to (5.700 and 4.200) shoot/explant when 2mgL^{-1} Kin + 0.4mgL^{-1} IBA and 1mgL^{-1} Kin + 0.4mgL^{-1} IAA respectively, when added to the MS medium compared with all treatment except the treatment 1mgL^{-1} Kin + 0.4mgL^{-1} IBA. Whereas, the less number of shoots (1.500) was found on MS medium free hormones. As it is illustrates from the same table (1) that the effect of different concentration of Kin separately, IBA and IAA together on number of leaves, number of node and shoot length. The maximum number of leaves and number of nodes (20.100 leaves/ explant and 5.100 node/ explant) were obtained on MS medium supplemented with 3mgL^{-1} Kin. While the highest length of shoots (4.41cm) was found when 2mgL^{-1} Kin added to the MS medium. However, the minimum number of leaves, number of nodes and shoot length (10.500 leaves/ explant 2.200node/explant and 1.55cm) respectively, were found on MS medium free hormones. About the combinations between Kin concentrations with IBA or IAA produced of number of leaves; number of nodes and shoot length were increased on the combinations. The maximum number of leaves (19.80 and 17.30) leaves /explant when (2mgL^{-1} Kin + 0.4mgL^{-1} IBA) and (1mgL^{-1} Kin + 0.4mgL^{-1} IAA) were added to the MS medium, respectively. While the minimum number of leaves (13.900 and 13.800 leaves/ explant) was found on MS medium supplemented with 2mgL^{-1} Kin + 0.2mgL^{-1} IBA and

3mgL^{-1} Kin + 0.4mgL^{-1} IBA respectively. On the other hand, the interaction also revealed significant difference and the treatment of Kin at 2mgL^{-1} + IBA at 0.4mgL^{-1} gave the highest number of nodes (5.100 node/explant). However, the interaction between Kin concentrations with IBA or IAA concentrations gave the maximum number of nodes (4.00 node/explant) was obtained when Kin at 1mgL^{-1} + 0.4mgL^{-1} IAA was added to the MS medium. Whereas, the less number of node (2.80 node/ explant) was observed on MS medium containing 1mgL^{-1} Kin + 0.2mgL^{-1} IBA. The results clarify that the shoot length was significantly affected when using the interaction between Kin with IBA or IAA. While, using MS medium containing 3mgL^{-1} Kin + 0.4mgL^{-1} IBA and 3mgL^{-1} Kin + 0.2mgL^{-1} IAA which gave highest length of shoot (3.85 and 3.91) cm was more affected increased significantly the shoot length when compared with majority and control treatments which gave the lower shoot length. Treatment which gave minimum length of the shoots length was observed on MS medium plus 1mgL^{-1} Kin + 0.2mgL^{-1} IBA which amounted to (2.030 cm). The figure (1) illustrates the effect of different concentrations of Kin, IBA and IAA at multiplication stage after 8 weeks of culture.

Similar response was observed with *Tiliaplathyphyllosscop* (Üçler *et. al.* 2001). The minimum number were formed with MS medium free growth regulators (control) and MS medium having 3mg L^{-1} Kin in tissue cultures (in addition to in integral plants and plant organs), cytokinins seem to be necessary for plant cell separation. Cytokinins are very active in encouraging direct or indirect shoot development.

Table (1): Effect of Kin alone, IBA and IAA combination with Kin on multiplication of photinia explant culture on MS medium after 8 weeks.

PGRs	PGRs Conce. Mgl ⁻¹	Shoot Number	Leaves Number	Node Number	Shoot Length (cm)
control		1.500e	10.500d	2.200e	1.550d
Kin	1	2.600de	13.600cd	3.100cde	3.480ab
Kin	2	4.100bc	15.400abcd	3.000cde	4.410a
Kin	3	6.00a	20.100a	5.100a	3.120bc
Kin+IBA	1+0.2	3.100cd	14.100cd	2.800de	2.030cd
Kin+IBA	2+0.2	3.700cd	13.900cd	3.600bcd	2.240cd
Kin+IBA	3+0.2	4.200bc	15.700abc	4.100abcd	3.620ab
Kin+IBA	1+0.4	5.200ab	17.600abc	4.600ab	3.590ab
Kin+IBA	2+0.4	5.700a	19.800ab	5.100a	3.700ab
Kin+IBA	3+0.4	4.200bc	13.800cd	4.300abc	3.850ab
Kin+IAA	1+0.2	3.300cd	14.500cd	4.000abcd	2.870bc
Kin+IAA	2+0.2	3.400cd	16.100abc	3.200bcde	2.030cd
Kin+IAA	3+0.2	3.100cd	14.300cd	3.900abcd	3.910ab
Kin+IAA	1+0.4	4.200bc	17.300abc	4.000abcd	3.670ab
Kin+IAA	2+0.4	4.100bc	15.100abcd	3.700abcd	2.820bc
Kin+IAA	3+0.4	3.500cd	14.900bcd	3.200bcde	2.180cd

* Means followed by the same letter within each character (column) do not differ significantly ($P \leq 0.05$) according to Duncan's Multiple Range Test (Duncan, 1955)



Figure. (1): Multiple shoot regeneration of *Photinia x fraseri* on MS medium supplemented with (1) 3 mg⁻¹ Kin. (2) 2mg⁻¹Kin+ 0.4mg⁻¹ IBA. (3) 2mg⁻¹ Kin + 0.4mg⁻¹ IAA.

Effect of different medium with the fixed and best concentration of Kin+IBA and Kin+IAA on the average shoot number and leaves number /explant of Photinia explant (node segment and shoot tips).

Table (2) showed the effect of different type of media supplemented with fixed concentration of (Kin at 2mg⁻¹ +0.4mg⁻¹ IBA and Kin at 1mg⁻¹+0.4mg⁻¹ IAA) on shoot number and leaves number from two types of explant (node segment and shoot tips).

Regarding means value of explants show, the maximum number of shoots (4.39 shoots/explant) from node segment was significant compared with shoot tips (3.88 shoot/ explant). While the means value of type of media plus PGRs at fixed concentration, the maximum

number of shoots (5.75) shoot/ explant was significantly increased when the explant cultured on WPM medium plus Kin +IBA compared with all treatments. While the highest number of shoot (4.60) was observed on WPM medium containing Kin+IAA but the increase was not significantly. Whereas, the minimum number of shoots (3.15) was found on White medium supplemented with Kin at 1 mg⁻¹+IAA at 0.4 mg⁻¹respectively.

The interaction between the explant and types of medium containing fixed concentration of Kin+ IBA and Kin+ IAA was significantly increased shoots number. The highest number of shoots (5.90) was recorded in node segment when cultured on WPM containing Kin+ IBA and it was increased significantly compared with

all treatments from shoot tip in control treatment when shoot tip cultured on WPM containing Kin+ IBA. In addition the maximum number of shoot (5.60) was observed for the shoot tip cultured on the same medium. While, the least number of shoot (3.00) was obtained from shoot tip when cultured on White medium containing Kin+ IAA.

On the other hand, the mean values of experimental results regarding on the same table, the highest number of leaves from node segment and shoot tip were (15.6 and 13.98) respectively, the node segment was increased significantly compared with shoot tip. The maximum number of leaves (20.10) was recorded on WPM medium supplemented with Kin+ IBA and it was increased significantly compared with all treatments which containing different media plus Kin +IBA and Kin+IAA. Whereas, the minimum

number of leaves (13.10) were found on B₅ and DKW media containing Kin +IAA.

The combinations between the explant and types medium containing fixed concentration of Kin+ IBA and Kin+ IAA to leaves emergence show that the maximum number of leaves emergence from node segment (20.60) and from shoot tip (19.60) was found on WPM medium supplemented with Kin+ IBA and this treatments was significantly increased with all treatment for shoot tip and some treatments for node segments when culture on WPM, B₅, DKW and White media containing Kin+ IBA. However, the less number of leaves (12.70) was obtained from shoot tips when cultured on white medium containing Kin+ IBA. The figure (2) illustrates the effect of different type of media with fixed concentrations of Kin, IBA and IAA on node segment and shoot tips at multiplication stage after 8 weeks of culture.

Table (2): Effect of different type of media with fixed concentration of Kin+ IBA and Kin+ IAA on the average shoot number and leaves number /explant of *Photinia* explant (node segment and shoot tips) on multiplication stage after 8 weeks.

PGRs	Media	Shoot Number		Means Type of Media with PGRs	Leaves Number		Means Type of Media with PGRs
		Node seg.	Shoot tip		Node seg.	Shoot tip	
Kin+IBA	WPM	5.90a	5.60ab	5.75a	20.60a	19.60a	20.10a
	B ₅	4.80abc d	4.00cde	4.40bc	16.10ab	13.50b	14.80b
	DKW	4.20bcd e	3.70de	3.95bcd	16.20ab	13.10b	14.65b
	White	3.50de	3.40de	3.45cd	14.40ab	12.70b	13.55b
Kin+IAA	WPM	5.30abc	3.90cde	4.60b	17.90ab	13.30b	15.60b
	B ₅	3.80de	3.10e	3.45cd	13.40b	12.80b	13.10b
	DKW	4.30abc d	4.30abc d	4.30bc	12.80b	13.40b	13.10b
	White	3.30de	3.00e	3.15d	13.40b	13.40b	13.40b
Means of Explants		4.39a	3.88b		15.60a	13.98b	

* Means followed by the same letter within each character (column) do not differ significantly ($P \leq 0.05$) according to Duncan's Multiple Range Test (Duncan, 1955)

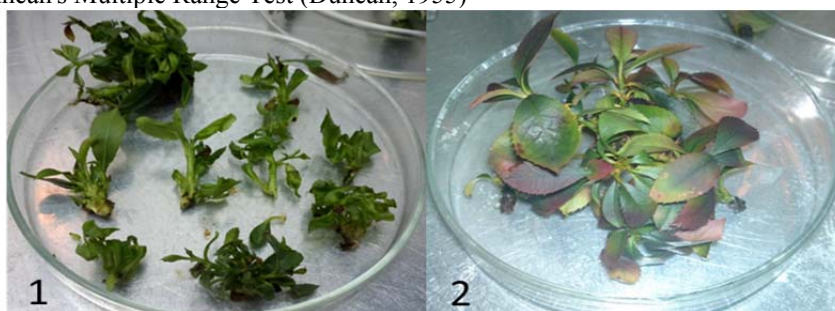


Figure. (2): Multiple shoot regeneration on the average shoot number and leaves number /explant of *Photinia x fraseri* explant (node segment on WPM medium supplemented with (1) 2mg^l⁻¹ Kin+ 0.4 mg^l⁻¹ IBA. (2) 2mg^l⁻¹ Kin + 0.4mg^l⁻¹ IAA.

Effect of different medium with the fixed and best concentration of Kin+ IBA and Kin+ IAA the average node number /explant and shoot length (cm) of Photinia explant (node segment and shoot tips).

It is shown from Table (3) the effect of different type of media supplemented with fixed concentration of (Kin at 2mg l^{-1} + 0.4mg l^{-1} IBA and Kin at 1mg l^{-1} + 0.4mg l^{-1} IAA) on number of node and shoot length form two types of explant (node segment and shoot tips).

Regarding means value of explants show; the maximum number of node (4.40node/ explant) from node segment was significant compared with shoot tips (3.79node/ explant). While the means value of type of media plus PGRs at fixed concentration, the maximum number of nodes(5.70) shoot/ explant were significantly increased when the explant cultured on WPM medium plus Kin +IBA compared with all treatments. While the highest number of node (5.00) was observed on WPM medium containing Kin+ IAA but the increased was not significantly. Whereas the minimum number of node (3.15) was found on White medium supplemented with Kin +IBA and Kin +IAA respectively.

The interaction between the explant and types of medium containing fixed concentration of Kin+ IBA and Kin+ IAA was significantly increased. The highest number of shoots (6.40) was recorded in node segment when cultured on WPM containing Kin+ IBA and it was increased significantly compared with all treatments from shoot tip. In addition the maximum number of node (5.00) was observed from shoot tip when the shoot tip cultured on the same medium. While, the lest number of node (3.00) was

obtained from shoot tip when cultured on White medium containing Kin+ IAA.

On the other hand, the mean values of experimental results regarding on the same table, the highest length of shoots from node segment and shoot tip (3.42 and 4.09) cm respectively, the shoot tip was increased significantly compared with node segment. While the mean values of different type media containing fixed concentration of Kin+ IBA and Kin+ IAA. The maximum length of shoots (4.79cm) was recorded on WPM medium supplemented with Kin+ IBA and it was increased significantly compared with all treatments which containing different media plus Kin +IBA and Kin + IAA. Whereas, the minimum length of shoots (3.05cm) were found on white media containing Kin+ IAA.

The combinations between the explant and types medium containing fixed concentration of Kin+ IBA and Kin+ IAA length emergence shows that the highest length of shoots emergence from shoot tip (5.28cm) and from node segment (4.31cm) was obtained on WPM medium improved with Kin+ IBA and this treatments was significantly increased with all treatment for node segment and some treatments for shoot tip when culture on WPM, B₅, DKW and White media containing Kin+ IBA. However, the less number of leaves (3.05cm)were obtained from node segment and shoot tips when cultured on while medium plus Kin+ IBA and Kin +IAA. The figure (3) illustrates the effect of different type of media with fixed concentrations of Kin, IBA and IAA on node segment and shoots tips at multiplication stage after 8 weeks of culture.

Table (3): Effect of different type of media with fixed concentration of Kin+ IBA and Kin+ IAA on the average node number /explant and shoot length (cm) of *Photinia* explant (node segment and shoot tips) on multiplication stage after 8 weeks.

PGRs	Media	Node Number		Means Type of Media with PGRs	Shoot Length (cm)		Means Type of Media with PGRs
		Node seg.	Shoot tip		Node seg.	Shoot tip	
Kin+IBA	WPM	6.40a	5.00bc	5.70a	4.31abc	5.28a	4.79a
	B5	4.80bcd	4.50bcd e	4.65bc	3.21bc	4.54ab	3.88bc
	DKW	4.50bcd e	3.30de	3.90cd	3.32bc	4.33abc	3.83bc
	White	3.20e	3.10e	3.15d	3.05c	4.51ab	3.78bc
Kin+IAA	WPM	5.80ab	4.20cde	5.00ab	3.97abc	4.41abc	4.19ab
	B5	3.90cde	3.60cde	3.75cd	3.21bc	3.43bc	3.32bc
	DKW	3.60cde	3.30de	3.45d	3.24bc	3.24bc	3.24c
	White	3.00e	3.30de	3.15d	3.05c	3.05c	3.05c
Means of Explants		4.40a	3.79b		3.42b	4.09a	

* Means followed by the same letter within each character (column) do not differ significantly ($P \leq 0.05$) according to Duncan's Multiple Range Test (Duncan, 1955)

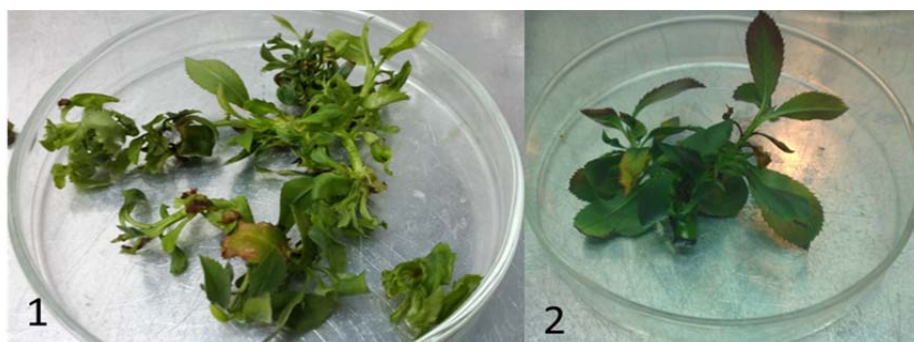


Figure. (3): Multiple shoot regeneration the average node number /explant and shoot length (cm) from shoot tips of *Photinia x fraseri* explant (node segment) on WPM medium supplemented with (1) 2mg/l-1 Kin+ 0.4 mg/l-1 IBA. (2) 2mg/l-1 Kin + 0.4mg/l-1 IAA.

Effects of salt strength of MS media and different concentrations of IAA, IBA and NAA on rooting stage

1. Rooting percentage

The percentage of root was significantly affected by the various treatments tested on photinia (Table 4) The mean value of type of auxin show that the highest percentage of root formation when NAA used and gave the better results on root percentage formation (98.33%) than IBA and IAA. While the mean value of type media, the percentage of root on (84.29%) was detected as a result of amending the $\frac{1}{4}$ MS medium compared with $\frac{1}{2}$ MS medium (81.5%) and it was significantly increased. However, the mean values of auxin concentrations show that the highest percentage of root development

(100%) was observed when 0.8mg/l add to the medium. A similar results was reported by (Danial *et. al.* 2008) by using of MS medium with (0.5mg/l⁻¹)NAA encouraged root growth (5.67 ± 1.15) and MS medium containing 1mg/l⁻¹IBA gave (2.15 ± 1.02) of *Dianthus Caryophyllus L* culture.

The effect of the three interactions (diverse media concentrations, various kinds of auxine and their concentrations) showed that rooting percentage could be achieved with the use of basic $\frac{1}{4}$ MS medium and $\frac{1}{2}$ MS medium increased with NAA, IBA and IAA concentrations. The explants of *photinia* cultured on $\frac{1}{4}$ MS medium supplemented with (0.2, 0.6 and 0.8)mg/l⁻¹NAA and (0.6 and 0.8) mg/l⁻¹IBA concentration produced an average of (100%) rooting compared with $\frac{1}{2}$ MS medium

supplemented with all concentration of NAA, IAA and IBA concentration. Oppositely, the lowest percentage of roots were made on

explants (42.5%) with $\frac{1}{4}$ MS and $\frac{1}{2}$ MS medium without hormones.

Table (4): Effect of salt strength of MS media and different types of auxin concentrations on roots response of *photinia* after 8 weeks.

Auxins Type	Conce. Mg ^l ⁻¹	% Root		Means of Auxinsconce	Means of Auxins Type
		$\frac{1}{2}$ MS	$\frac{1}{4}$ MS		
Control	0	42.5f	42.5f		
IAA	0.2	77.5de	85abcde	81.25c	81.25a
IAA	0.6	75e	87.5abcde	81.25c	
IAA	0.8	80cde	82.5bcde	81.25c	
IBA	0.2	80cde	95abc	87.5bc	90.83a
IBA	0.6	80cde	100a	90abc	
IBA	0.8	90abcde	100a	95ab	
NAA	0.2	92.5abcd	100a	96.25ab	98.33a
NAA	0.6	97.5ab	100a	98.75a	
NAA	0.8	100a	100a	100a	
Means of Media		81.5b	84.29a		

* Means followed by the same letter within each character (column) do not differ significantly ($P \leq 0.05$) according to Duncan's Multiple Range Test (Duncan, 1955)

2. Roots number

As presented in Table (5) Fig (4,5), data shown that there was a significant variance in the number of roots formed on shootlets of *photinia* as a consequence of tested treatments. The mean value of type of media show that the highest number of roots/shootlet (4.76) was recorded with $\frac{1}{4}$ MS medium compared with $\frac{1}{2}$ MS medium (4.53) roots / shootlet and it was significant increasing auxin concentrations. However, the mean value of type of auxin show that the highest percentage of root formation when NAA used and gave the better results on root percentage formation (5.66) and significantly increased the number of root as is compared to IBA and IAA . Data concerning the mean result of auxin concentration treatments and it's the maximum number of roots/shootlet (6.05) was recorded for shootlet with 0.6 mg^l⁻¹NAA, while, the lowest value (2.50) was recorded 0.6 mg^l⁻¹IAA.

Consequences regarding the effect of the three factors (diverse salt of MS medium concentrations, kinds of auxin and auxin concentrations) showed that the roots /shootlet could be found with the use of unchanged $\frac{1}{2}$ MS medium and $\frac{1}{4}$ MS medium complemented with NAA, IAA and IBA concentrations. Data also

indicated that IBA and NAA concentration when extra to the $\frac{1}{4}$ MS has the pronounced and significant consequence on this parameter. Shootlet from *photinia* cultured on $\frac{1}{4}$ MS medium improved with 0.6 mg^l⁻¹NAA concentration formed the maximum number of roots/shootlet (6.5roots/shootlet) compared with all treatments having $\frac{1}{2}$ MS medium complemented with IAA, IBA and NAA on all concentration. Otherwise, the lowest number of roots on *photinia* explants (2.3 roots/ shootlet) and was recorded with $\frac{1}{2}$ MS medium having 0.2mg^l⁻¹IAA. Consequences under discussion are in harmony with those described by (Ali *et. al.* 2008). They explained that in order to develop *in vitro* adventitious rooting, the isolated plantlets were cultured on media having 0.1, 1.0 and 10.0 mg/l IAA or NAA in several physical conditions. Optimum adventitious rooting and succeeding plant survival was found by culturing plantlets in medium having 0.1 mg/l NAA for 8-16 weeks prior to transplanting to soil (Tisserat, 1982). Date palm plants may be obtained by transferring separate plantlet to MS medium supplemented with 0.1 mg/l NAA to improve rooting and 0.01mg/l BA to improve shoot structure(Omar.1988).

Table (5): Effect of salt strength of MS media and different types of auxin concentrations on average root number formed on shootlet of *photinia* after 8 weeks.

Auxins Type	Conce. Mgl ⁻¹	Number of Root		Means of Auxins conce.	Means of Auxins Type
		½MS	¼MS		
Control	0	2.6ef	2.6ef		
IAA	0.2	2.3f	2.7ef	2.5e	3.50b
IAA	0.6	3.6def	3.8de	3.7d	
IAA	0.8	4.2cd	4.4cd	4.3cd	
IBA	0.2	4.7bcd	4.7bcd	4.7bc	5.00a
IBA	0.6	4.9bcd	5.4abc	5.15abc	
IBA	0.8	4.6bcd	5.7abc	5.15abc	
NAA	0.2	5.4abc	5.7abc	5.55ab	5.66a
NAA	0.6	5.6abc	6.5a	6.05a	
NAA	0.8	4.7bcd	6.1ab	5.40ab	
Means of Media		4.53b	4.76a		

* Means followed by the same letter within each character (column) do not differ significantly ($P \leq 0.05$) according to Duncan's Multiple Range Test (Duncan, 1955).

3. Roots length (cm)

Data in Table (6) Fig (4,5) had been that the maximum mean value of root lengths (4.04 cm) were obtained due to modifying the ¼MS medium, although, the shortest roots (3.23 cm) were developed on the ½MS medium and it was significantly increased with increasing auxin concentrations. It is clear from the same Table (6) shown that the mean value of type of auxins were significant variances between IBA, NAA and IAA on rooting lengths of *photinia*. The longest root (4.62cm) was recorded in medium having NAA and significant changes compared with medium having IAA, and the longest root (3.77) was recorded in medium having IBA. In contrast, the mean result of auxin concentration treatments showed that the highest length of roots/ shootlets (5.05cm) was recorded medium (0.6mg l⁻¹), and the shortest roots (2.81cm) were found on medium 0.2mg l⁻¹ IAA.

Comparable results were found by (Manisha, *et. al.* 2001) on *Alnus nepalensis*, and (Gad, *et. al.* 1999) on *Khaya ivorensis*

The result of different media treatments and its interactions with diverse types of auxin on the three concentrations on *photinia* root length, that the kinds of auxin and concentration have no significant influence on this parameter. The interactions between cultured media and different types of auxins show that the ¼MS medium with 0.6 mg l⁻¹ NAA enhanced rooting and produced an average (5.29cm) compared to all treatments having ½MS medium. Conversely, the lowest length of root was made (2.04cm) with ½MS medium free auxin. The benefit of NAA over other auxins has also been reported in other plant species such as *Rosa Damascena* (Boskabady, *et. al.* 2011) *Rosa grussanteplitz* and *Rosa centifolia*. (Mirza, *et. al.* 2011)

Table (6): Effect of salt strength of MS media and different types of auxin concentrations on average root length (cm) of photinia after 8 weeks.

Auxins Type	Conce. Mgl ⁻¹	Root Length		Means of Auxinsconce	Means of Auxins Type
		½MS	¼MS		
Control	0	2.04g	2.28fg		
IAA	0.2	2.31fg	3.32cdef	2.815ef	
IAA	0.6	2.51efg	3.64cd	3.075e	3.02b
IAA	0.8	2.89defg	3.45cde	3.170ee	
IBA	0.2	2.87defg	4.78ab	3.825cd	
IBA	0.6	3.58cde	4.94ab	4.260bc	3.77ab
IBA	0.8	2.88defg	3.57cde	3.225de	
NAA	0.2	4.21abc	5.12ab	4.665ab	
NAA	0.6	4.81ab	5.29a	5.05a	4.62a
NAA	0.8	4.05bc	4.24abc	4.145bc	
Means of Media		3.23b	4.04a		

* Means followed by the same letter within each character (column) do not differ significantly ($P \leq 0.05$) according to Duncan's Multiple Range Test (Duncan, 1955)

Acclimatization Stage

The successful transfer of plantlets from culture tubes to the soil is one of the most essential steps in a sexual micro propagation program of several plant species. The plantlets were transferred from the *in vitro* conditions to greenhouse location, Fig (6). After 8 weeks in

rooting medium, the rooted plantlets were washed under tap water to remove the remains of culture medium, which is a goal of microorganism's attacks because of its carbon source and agar content. The survival rate reached to 85% and after 2 weeks the plantlets transferred to greenhouse environments.



Figure. (4): Rooted shootlets of *Photinia x fraseri* on ¼ MS medium supplemented with 0.8 mg l⁻¹ IBA



Figure. (5): Rooted shootlets of *Photinia x fraseri* on ¼ MS medium supplemented with 0.6 mg l⁻¹ NAA



Figure.(6): Acclimatized plantlets of *Photinia x fraseri* in the greenhouse (8 weeks after potting).

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زیده کرنا رووه کی (Photinia x fraseri) دهر فیهی له شی زیندی بکارینانا جهند بیافین جوآر جوآر

پوخته:

بلندترین ژمارا چهقا پهیدا بون ژ گریکین رووه کی فیتونیا *Photinia x fraseri* نهوین هاتینه جانندن لسه بیافی MS نهوی بیگ هاتی ژ جهند ریژیت جوآر جوآر ژ کایتینی Kin بتنی (۱، ۲ و ۳) ملغم / لتر و ههر وهسا دگهل جهند ریژیت جوآر جوآر یه IAA و IBA . نهنجام دیار بون کو ریژیت Kin بتنی و گهل IBA و IAA زیده کرنه کا بهر جاف هه بو وژمارا چهقا که هشته (۶ چهقا/ پارچه کین رووه کی) و نهف زیده کرنه یا بهر جاف بو بهراوه دگهل کارلیکین دی. ههر وهسا باشترین نهنجام هاتنه تومار کرن یه ژمارا چهقا و ژمارا بهلگا لدهمی (گریک و پشکیت) رووه کی فیتونیا هاتیه جانندن. بلندترین ژمارا چهقا (۵.۹۰ چهقا/ پارچه کا رووه کی) هاتنه تومار کرن لدهمی گریک هاتینه جانندن لسه بیافی WPM یی کو ریژه کا تیراتیایه جیکیر یا Kin+ IBA و نهو زیده کرنه یا بهر جاف بو بهراوه دگهل کارلیکین دی یه سهریت پشکا. ههر وهسا بلندترین ژمارا بهلگا (۲۰.۱۰ بهلگ / پارچه کا رووه کی) هاتنه تومار کرن لدهمی رووه ک هاتیه چانندن لسه بیافی WPM یی کو Kin+ IBA و نهو زیده کرنه یا بهر جاف بو بهراوه دگهل کارلیکیندی.

رووه کین هاتینه چانندن لسه $MS^{1/4}$ یی کو ۰.۶ ملغم/ لتر NAA بلندترین ژمارا ره هاتنه جانان و گه هشته (۶.۵ رهه/ بارچه کا رووه کی) و نهو زیده کرنه یا بهر جاف بو بهراوه دگهل کارلیکیندی نهوین لسه بیافی $MS^{1/2}$ یی کو ههمی جورین اوکسیناتا تیدا و دگهل ریژین وان. و ههر وهسا دریزترین ره هاتنه تومار کرن لسه بیافی $MS^{1/4}$ یی کو ۰.۶ ملغم/ لتر NAA و ریژا وان که هشته (۵.۲۹ سم). ههمی رووه کین ره داین هاتنه فه گوهاستن بو ناف بیافی ناخی و بلندترین ژماره

مانه ساخ

تضاعف نبات الفيتونيا (*Photinia x fraseri*) خارج الجسم الحي باستعمال اوساط غذائية مختلفة

الخلاصة:

أعلى معدل من الافرع تم الحصول عليها من العقد لنبات الفيتونيا *Photinia x fraseri* المزروعة على وسط MS المزود بتركيز مختلفة من Kin لوحده (1، 2، و 3) ملغم / لتر. اظهرت النتائج بان مستويات الـ Kin لوحده و تداخله مع IBA و IAA اثرت معنويا على عدد الافرع. عدد الافرع / جزء نباتي (6) فرع/ جزء نباتي عند اضافة 3 ملغم/ لتر من Kin وكان التفوق معنوي مع تراكيز اخر من الـ Kin. بينما تأثير اضافة تراكيز ثابتة من Kin+ IBA و Kin+ IBA الى اوساط غذائية مختلفة كان واضحا على معدل عدد الافرع و عدد الاوراق لنبات الفيتونيا (العقد والقمم النامية). اكبر عدد من الافرع بلغ (5.90 فرع / جزء نباتي) سجلت من العقد عندما زرعت على WPM المزود بتركيز ثابت من Kin+ IBA وكانت الزيادة معنوية مقارنة بجميع المعاملات التي زرعت فيها القمم النامية. بينما اكبر عدد من الاوراق (20.10 ورقة/ جزء نباتي) سجلت من الاجزاء النباتية التي زرعت على وسط WPM المزود بـ Kin+ IBA وكانت الزيادة معنوية مقارنة بجميع المعاملات الاخرى. النباتات التي زرعت على وسط MS^{1/4} واجهت بـ 0.6 ملغم/ لتر NAA انتجت اعلى عدد من الجذور بلغ (6.5 جذر/ جزء نباتي) مقارنة بجميع المعاملات الاخرى التي احتوت MS^{1/2} والمزود بكافة الاوكسينات وبجميع تراكيزها. بينما اعلى معدل لطول الجذور ظهرت عند زراعة النباتات على وسط MS^{1/4} حاوي على 0.6 ملغم/ لتر NAA والتي بلغ معدلها (5.29 سم) مقارنة بجميع المعاملات الاخرى التي احتوت على وسط MS^{1/2}. جميع النباتات نقلت وبنجاح الى التربة بعد اجراء عملية التقسية مع تسجيل اعلى معدل للبقاء على قيد الحياة.