EFFECT OF DIFFERENT CONCENTRATION OF BA AND IAA ON MICROPROPAGATION OF *Gardenia jasminoides*

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ABSTRACT

The goal of this study was to use plant tissue culture technique in vegetative propagation of Gardenia (*Gardenia jasminoides*) by using single nodes and shoot tips excised from soft cuttings and treated with different concentrations of growth regulators. The results revealed that the use of different disinfistants was highly effective in reducing cultures contamination. The use of mercuric chloride (0.1%, $HgCl_2$) for 10 minutes was very effective in preventing contamination and gave the highest survival percentage (99%). Results at initiation stage revealed that the culture of single nodes of Gardenia on a medium containing 3 mgl^{-1} BA+ 0.9 mgl^{-1} IAA gave the best growth response in which highest number of shoots and leaves. At vegetative multiplication stage, the results showed that the medium supplemented by 1.5 and 4.5 mgl^{-1} BA+ 0.6 mgl^{-1} IAA gave the highest value of growth length and gave the highest number of shoots per explant. In order to promote the shoots produced at vegetative multiplication stage to root, it was noticed that the treatment of 4 mgl^{-1} NAA and8 mgl^{-1} IAA gave the highest percentage of rooting and values of the number and length of roots. Plantlets obtained were transferred to pots and acclimatized with 95 % success.

INTRODUCTION

Gardenia jasminoides or common Gardenia is a member of the family Rubiceae and belongs to the genus Gardenia. There are over than 200 species of Gardenias. Two species are of primary importance, *Gardenia jasminoides,* containing many cultivars, and *Gardenia thunbergia*, grown primarily as a rootstock. Gardenia is an evergreen shrub, which can grow up to 2 - 2.6 m tall and spread about the same .They have glossy, leathery and dark green leaves. Depending on the cultivar, the flowers can be either single or double and up to 10 cm in diameter. They are waxy, white and very fragrant . Gardenias can be used as screens, hedges, borders or ground covers. They may also be used as free-standing specimens or in mass plantings. The most important classes of the plant growth regulators used in tissue culture are the auxins and cytokinins. The relative effects of auxin and cytokinin ratio on morphogenesis of cultured tissues were demonstrated by Skoog and Miller (1957) and still serve as the basis for plant tissue culture manipulations today. Salman *et al*. (1994) while propagating *Citrus aurantium* rootstocks *in vitro* by culturing lateral and terminal buds (taken from seedlings) on MS medium enriched with different BA and GA_3 concentrations, found that the response of terminal buds was better than the lateral buds on MS medium supplemented by 2 mgl^{-1} BA, 0.2 mgl^{-1} GA_3 and 80 mgl^{-1} adenine sulphate. Researchers referred to the importance of BA addition to culture medium with concentrations of 0.2- 3 mgl^{-1}, Seyhan and Ozzambak (1994) found

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that using of BA (0.5 mgl^{-1}) led to the best growth of buds of two olive cultivars (Memecik and Domat) during 35- 40 days from culture. In gardenia, Al-Juboory *et al*. (1998) found that a comparison of cytokinins activities showed that the best shoot proliferation was obtained from gardenia leaves treated with benzylaminopurine (BA), N-phenyl–N-1,2,3-thidiazol-5yl urea (TDZ) and zeatin as compared with kinetin and 2iP and the longest shoots were produced in cultures grown with either BA or 2iP.Similar results on gardenia were recorded by Pontikis,1983; Economou and Spanoudaki,1986 and George *et al*,1993. All showed that BA alone has proved superior to 2iP and kinetin for promotion of axillary bud development from shoot tip and *in vitro* derived shoot explants of gardenia. Abdullah *et al* (2003) have stated that many plantlets were obtained by culturing shoot cuttings of gardenia in MS nutrient media, supplemented by BA and IAA.

The objectives of this study were: 1- To propagate *Gardenia jasminoides* by tissue culture technique using lateral and terminal buds as explants.

2- To evaluate the different type and concentrations of cytokinins (BA) and auxins (NAA and IAA) alone or in combination on shoot initiation, shoot proliferation and root formation on shoots proliferated *in vitro*.
3- To evaluate the effect of salt strength of MS medium on *in vitro* rooting of Gardenia shoots.

MATERIALS AND METHODS

1. Plant Materials [Source of Explants]: Actively growing shoots, 10-20 cm long were cut from 3years old *Gardenia jasminoides* grown in the greenhouse of Horticulture Dept/College of Agriculture at Dohuk University. Immediately after collection, the shoots were kept in polyethylene bags and taken to the laboratory.

2. Explant Preparation: Shoots were stripped of their leaves and washed in tap water for 60 minutes to remove soil and other superficial contamination, followed by tap water and liquid soap for 20 minuets, followed by three – five minute rinses in sterile distilled water. Then they were cut into shorter sections 1.5 cm long including [terminal (apical) bud] and single nodes with axillary bud .To inhibit tissues browning the shoot sections were placed in cold antioxidant solution containing (150 mgl^{-1}) citric acid and (100 mgl^{-1}) ascorbic acid for 30 minutes followed by 5 minute rinses in sterilized distilled water (Olivares *et al*, 1990).

3. Explants Disinfestation: Shoot tips and nodes with axillary buds were removed and disinfested by immersion in solutions of the following compounds:

A. Sodium Hypochlorite (NaOCl), commercial bleach solution containing 5% sodium hypochlorite, was used in 1, 2 and 3% v/v for 5 and 10 minutes.

B. Ethyl Alcohol (C_2H_5OH),

1. Concentration of 70% for 5, 10 and 15 minutes
2. Concentration of 75% for 5, 10 and 20 minutes

C. Mercuric Chloride ($HgCl_2$), (0.05%- 0.1%) w/v for 2.5, 5, 7.5 and 10 minutes.

The disinfested tissues [explants] were rinsed 3-4 times with sterilized distilled water, and the ends of explants exposed to sterilant were trimmed.

Culture Initiation Stage: Effect of BA Concentration on Explants Establishment: Benzyl adenine (BA) with 0, 1.5, 3 and 4.5 mgl^{-1} were added to the culture medium to observe the response of cultured explants. Ten explants were

cultured (an explant in each test tube for each concentration). They were incubated on 24±1°C under light conditions of 16 light hours and 8 darkness hours. The results were recorded after 4-6 weeks from planting.

Effect of the Interaction between BA and IAA on Explants Establishment: Different concentrations of BA and IAA were tested to find out their effect on culture initiation when combined together. BA was used at 0, 1.5, 3 and 4.5 mgl^{-1} and IAA at 0, 0.3, 0.6 and 0.9mgl^{-1}. Ten test tubes were used for each treatment on 24±1°C under light conditions of 16 light hours and 8 darkness hours.

Multiplication Stage: Effect of BA on Multiplication Stage: BA was tested in 0, 1.5, 3 and 4.5 mgl^{-1} to discover its effects on number and length of vegetative growth.

Effect of the Interaction between BA and IAA on Multiplication Stage: BA was added in 0, 1.5, 3 and 4.5 mgl^{-1} and IAA in 0, 0.2, 0.4 and 0.6 mgl^{-1} to reveal their effect on culture multiplication when combined together. GA_3 was added to MS medium with 3mgl^{-1} to all the treatments including control treatment. The parameters investigated in the experiments in stage1 and stage 2 were the same, that is,

1- Percentage of explants that exhibit normal growth.
2- Shoot number /explant.
3- Length of shoots (cm).
4- Leaves number /explant.

This evaluation was performed on a weekly basis for 4-6 consecutive weeks. At the end of the six week, the results were compiled, averaged and expressed as a percentage or number for each treatment.

Rooting Stage: Effect of Auxins on Rooting: The effect of IAA, IBA and NAA added to the culture medium on shoots rooting was studied by carrying out several separate experiments by adding IBA, NAA with (0,1, 2, 3 and 4) mgl^{-1} and IAA with (0,2,4,6 and 8) mgl^{-1} .All these treatments were examined in both full and half salt strength medium.

Acclimatization Stage: After 6-8 weeks from Gardenia shoots rooting, several plantlets were selected from those that formed good vegetative and seedy growth. They were washed under tap water to remove agar from the roots which might be a source of contamination. It is important to avoid cutting of any part of the roots during washing. They were then put in Benlate fungicide solution (0.1%) and then planted in plastic pots filled with a sterilized mixture of peat moss and river soil (2:1). In order to maintain high humidity in culture environment, the pots were covered with a light plastic cover which permits light passing and contains many openings to permit air entrance. Plants were watered and given a solution containing MS salts with 0.25 of the salt strength. The plastic cover was removed from time to time after two weeks from planting. After four weeks, the transplants were transplanted after being sprayed with Benlate fungicide (0.1%) as required.

Statistical Analysis: Experiments were carried out using Complete Randomized Block design (CRBD) with three factors (explant type X Cytokinin concentrations X Auxin concentrations) except in rooting stage in which there were two factors (full and half strength MS saltsX5 auxin concentrations). Significant differences among mean values were separated by using Duncan multiple range tests at $P \leq 0.05$ (Duncan, 1955).

RESULTS AND DISCUSSION

INITIATION STAGE: Effect of BA and IAA concentrations on explant establishment. Tables (1,2 and 3) shows using concentrations of BA (3 mgl^{-1}) led to obtain the highest number of shoots (2.60 shoots/explant) and leaves (2.73 leaves/explant) and shoot length (2.21 cm). This declares the necessity of cytokinin (BA) presence in initiation medium. This fact has been discussed in many published studies in tissue culture of many fruit trees like pears (Hirabayashi *et al*, 1987), plum (Druart and Gruselle, 1986) and walnut (Penuela *et al*, 1987). Significant interactions were recorded between the types of buds and BA levels. The highest values for all the mentioned charactors were observed from the treatment of terminal buds with 3mgl^{-1}BA. Significant differences were observed between the terminal and lateral buds in which terminal buds produced more leaves and more shoot lengths, however, no significant differences were observed in case of the number of new shoots. Concerning the interaction between BA ,IAA and type of buds , it is clear that for lateral buds, the treatment of 3 mgl^{-1}BA+ 0.9 mgl^{-1} IAA gave the highest values of number of shoots (2.8 shoots/ explant) and number of leaves (3.5 leaves/explant). Whereas for average length of shoots, the treatment of 4.5 mgl^{-1} BA + 0.6 mgl^{-1} IAA gave the highest value of growth length (2.3 cm) in which significantly differed from the control treatment. In terminal buds, 3 $mgl^{-1}$of BA+ 0.9 mgl^{-1} IAA resulted in producing more branches (2.8 shoots/explant),for the average number of leaves ,3 mgl^{-1} of BA + 0.3 mgl^{-1} of IAA was superior on other treatments which gave an average of 3.4 leaves/explant . However, the average length of new shoots was significantly increased by the treatment of 3 mgl^{-1} BA+ 0 mgl^{-1}IAA. These results are in agreement with what have been found by (Singh *et al*,1994), that using of cytokinins and auxins in this category is very important and the role of cytokinins at this stage is essential to break apical dominance in buds and induce subsidiary meristem grown into shoots.

Table (1): The effect of BA concentrations and their interactions with IAA levels on the average number of new shoots of lateral and terminal buds at initiation stage.

Type of buds	BA mgl^{-1}	IAA mgl^{-1}				Type of buds X BA mgl^{-1}
		0	0.3	0.6	0.9	
Lateral	0	1.30 g	1.70 d-g	1.60 efg	2.00 b-g	1.65 e
	1.5	2.20 a-f	2.20 a-f	2.00 b-g	2.30 a-e	2.18 cd
	3	2.10 a-f	2.50 abc	2.70 ab	2.80 a	2.53 ab
	4.5	2.30 a-e	2.30 a-e	2.20 a-f	2.10 a-f	2.23 bc
Terminal	0	1.30 g	1.90 c-g	2.00 b-g	1.70 d-g	1.73 e
	1.5	2.40 a-d	2.00 b-g	2.20 a-f	2.40 a-d	2.25 bc
	3	2.70 ab	2.50 abc	2.70 ab	2.80 a	2.68 a
	4.5	2.20 a-f	2.00 b-g	1.80 c-g	1.50 fg	1.88 de

Type of buds	IAA mgl^{-1}				Effect of Type of buds
	0	0.3	0.6	0.9	
Lateral	1.98 a	2.18 a	2.13 a	2.30 a	2.14 a
Terminal	2.15 a	2.10 a	2.18 a	2.10 a	2.13 a

BA mgl^{-1}	IAA mgl^{-1}				Effect of BA mgl^{-1}
	0	0.3	0.6	0.9	
0	1.30 f	1.80 e	1.80 e	1.85 de	1.69 c
1.5	2.30 a-e	2.10 cde	2.10 cde	2.35 a-d	2.21 b
3	2.40 abc	2.50 abc	2.70 ab	2.80 a	2.60 a
4.5	2.25 b-e	2.15 cde	2.00 cde	1.80 e	2.05 b

* Means followed by the same letter within a column do not differ significantly (α=0.05) according to Duncan's Multiple Range Test (Duncan ,1955).

Table (2): The effect of BA concentrations and their interactions with IAA levels on the average number of leaves of lateral and terminal buds at initiation stage.

Type of buds	BA mgl^{-1}	IAA mgl^{-1}				Type of buds X BA mgl^{-1}
		0	0.3	0.6	0.9	
Lateral	0	1.00 l	1.70 g-l	1.30 kl	1.60 h-l	1.40 d
	1.5	1.50 i-l	2.30 c-i	2.60 c-f	2.10 d-k	2.13 c
	3	1.60 h-l	2.20 c-j	2.40 c-h	3.50 a	2.43 bc
	4.5	1.80 f-l	2.00 e-k	2.50 c-g	2.20 c-j	2.13 c
Terminal	0	1.00 l	2.00 e-k	1.30 kl	1.40 jkl	1.43 d
	1.5	2.70 b-e	2.20 c-j	2.50 c-g	3.00 abc	2.60 b
	3	2.80 a-e	3.40 ab	2.90 a-d	3.00 abc	3.03 a
	4.5	2.30 c-i	2.20 c-j	2.50 c-g	2.60 c-f	2.40 bc

Type of buds	IAA mgl^{-1}				Effect of Type of buds
	0	0.3	0.6	0.9	
Lateral	1.48 c	2.05 b	2.20 ab	2.35 ab	2.02 b
Terminal	2.20 ab	2.45 a	2.30 ab	2.50 a	2.36 a

BA mgl^{-1}	IAA mgl^{-1}				Effect of BA mgl^{-1}
	0	0.3	0.6	0.9	
0	1.00 g	1.85 ef	1.30 g	1.50 fg	1.41 c
1.5	2.10 cde	2.25 cde	2.55 bcd	2.55 bcd	2.36 b
3	2.20 cde	2.80 ab	2.65 bc	3.25 a	2.73 a
4.5	2.05 de	2.10 cde	2.50 bcd	2.40 bcd	2.26 b

* Means followed by the same letter within a column do not differ significantly (α=0.05) according to Duncan's Multiple Range Test (Duncan ,1955).

Table (3): The effect of BA concentrations and their interactions with IAA levels on the average length of new shoots (cm) of lateral and terminal buds at initiation stage.

Type of buds	BA mgl^{-1}	IAA mgl^{-1}				Type of buds X BA mgl^{-1}
		0	0.3	0.6	0.9	
Lateral	0	0.80 n	1.20 k-n	0.79 n	0.80 n	0.90 c
	1.5	1.10 lmn	1.80 g-j	1.90 f-j	2.00 e-i	1.70 b
	3	1.10 lmn	2.10 d-i	1.90 f-j	2.20 d-h	1.83 b
	4.5	1.20 k-n	1.80 g-i	2.30 c-g	2.10 d-i	1.85 b
Terminal	0	0.80 n	1.40 j-m	1.20 k-n	1.00 mn	1.10 c
	1.5	3.00 ab	2.40 c-f	2.20 d-h	2.50 b-e	2.53 a
	3	3.30 a	2.20 d-h	2.30 c-g	2.60 bcd	2.60 a
	4.5	2.80 abc	1.70 h-k	1.60 i-l	1.70 h-k	1.95 b

BA mgl^{-1}	IAA mgl^{-1}				Effect of BA mgl^{-1}
	0	0.3	0.6	0.9	
0	0.80 e	1.30 d	1.00 de	0.90 e	1.00 c
1.5	2.05 abc	2.10 abc	2.05 abc	2.25 ab	2.11 a
3	2.20 ab	2.15 abc	2.10 abc	2.40 a	2.21 a
4.5	2.00 abc	1.75 c	1.95 bc	1.90 bc	1.90 b

Type of buds	IAA mgl^{-1}				Effect of Type of buds
	0	0.3	0.6	0.9	
Lateral	1.05 c	1.73 b	1.72 b	1.78 b	1.57 b
Terminal	2.48 a	1.93 b	1.83 b	1.95 b	2.04 a

***** Means followed by the same letter within a column do not differ significantly (α=0.05) according to Duncan's Multiple Range Test (Duncan ,1955).

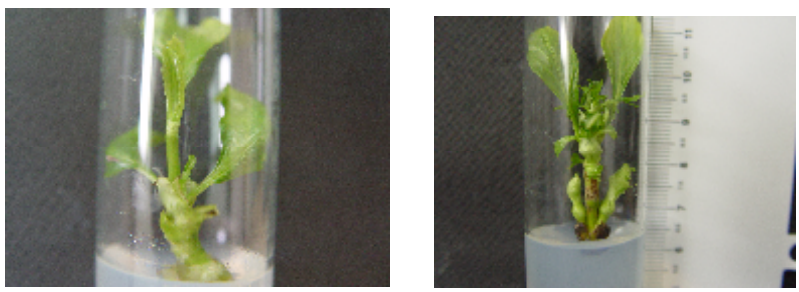


Image (1): Shoots initiation of *Gardenia jasminoides* on MS medium supplemented with BA+IAA at different concentrations after 4-6 weeks of culture

MULTIPLICATION STAGE: Effect of BA and IAA on shoot proliferation. Tables (4, 5 and 6) reveals the treatment of 3mgl^{-1} BA gave the highest rate of shoot number (3.06 shoots/ explant), leave number (3.39 leaves/explant) and shoot length (2.99 cm). It is thought that cytokinins promote the formation of woody tissues neighboring to the vascular tissues of the bud and stem, thus will make easy the translocation of water and nutrients which cause bud initiation (Mohammed and Al-Younis, 1991). These results agree with those reported by (Hammerschlag, 1982 and Brookner, 1991) in their studies on the importance of cytokinins in shoot multiplication. No significant differences in shoot number, leaves number and shoot length were recorded between the lateral and terminals buds tables (4,5 and 6). As for interaction between the type of bud and BA concentrations, the highest shoot number of new shoots (3.23 shoots/ explant), the highest number of leaves (3.55 leaves/explant) and highest average of length of new shoot (3.23 cm) were obtained when terminal buds were cultured in medium containing 3 mgl^{-1}BA. Interaction treatments for lateral buds revealed that the treatment of 4.5 mgl^{-1} BA + 0.6 mgl^{-1} IAA gave highest rate of the number of shoots (3.8 shoots/ explant), whereas the treatment of 3 mgl^{-1} BA+ 0.2 mgl^{-1} IAA gave the highest number of leaves (4.2 leaves/explant), and for shoot length, the treatment of 1.5 mgl^{-1} BA+ 0.6 mgl^{-1} IAA gave the highest value (3.4cm) which significantly differed from the other

treatments.For terminal buds, it could be noticed that the interaction of 3 mgl^{-1} BA + 0.4 mgl^{-1} IAA resulted in the production of more new shoots (3.6 shoots/explant). However more leaves (4.00 leaves/explant) and more shoot length (3.6cm) were obtained from the treatment of 3 mgl^{-1} BA + 0.6 mgl^{-1} IAA. An examination of the various media and growth regulator combinations that have been utilized for tissue culture of woody plant and fruit trees reveals that shoot proliferation both as terminal buds as well as axillary buds generally require the presence of both auxins and cytokinins (Skrivin, 1984). Furthermore many researchers have found that cytokinins ,especially BA, could stimulate axillary bud development ,but at high concentration, shoot elongation is suppressed (Da Silva *et al*. 2003) .These results are in line with many researchers ,who observed variables effects of cytokinins and auxin on shoot regeneration of many wood species.

Table (4): The effect of BA concentrations and their interactions with IAA levels on the average number of new shoots of lateral and terminal buds at multiplication stage. (3 mgl^{-1} of GA_3 were added to all the treatments).

Type of buds	BA mgl^{-1}	IAA mgl^{-1}				Type of buds X BA mgl^{-1}
		0	0.2	0.4	0.6	
Lateral	0	1.00 k	1.80 g-k	1.40 ijk	1.50 h-k	1.43 d
	1.5	1.20 jk	3.60 ab	3.20 a-e	2.70 b-g	2.68 b
	3	1.80 g-k	3.40 a-d	3.50 abc	2.90 a-f	2.90 ab
	4.5	2.00 f-k	3.60 ab	3.30 a-d	3.80 a	3.18 a
Terminal	0	1.30 jk	2.40 d-i	1.90 f-k	2.90 a-f	2.13 c
	1.5	2.20 e-j	2.80 a-g	2.50 c-h	2.70 b-g	2.55 bc
	3	2.60 b-g	3.20 a-e	3.60 ab	3.50 abc	3.23 a
	4.5	2.40 d-i	3.10 a-e	2.40 d-i	2.50 c-h	2.60 b

Type of buds	IAA mgl^{-1}				Effect of Type of buds
	0	0.2	0.4	0.6	
Lateral	1.50 d	3.10 a	2.85 ab	2.73 ab	2.54 a
Terminal	2.13 c	2.88 ab	2.60 b	2.90 ab	2.63 a

BA mgl^{-1}	IAA mgl^{-1}				Effect of BA mgl^{-1}
	0	0.2	0.4	0.6	
0	1.15 f	2.10 de	1.65 ef	2.20 cde	1.78 c
1.5	1.70 ef	3.20 ab	2.85 abc	2.70 bcd	2.61 b
3	2.20 cde	3.30 ab	3.55 a	3.20 ab	3.06 a
4.5	2.20 cde	3.35 ab	2.85 abc	3.15 ab	2.89 ab

*Means followed by the same letter within a column do not differ significantly (α=0.05) according to Duncan's Multiple Range Test (Duncan ,1955).

Table (5): The effect of BA concentrations and their interactions with IAA levels on the average number of leaves of lateral and terminal buds at multiplication stage. (3 mgl^{-1} of GA_3 were added to all the treatments).

Type of buds	BA mgl^{-1}	IAA mgl^{-1}				Type of buds X BA mgl^{-1}
		0	0.2	0.4	0.6	
Lateral	0	1.30 jk	1.80 h-k	1.70 ijk	2.20 f-j	1.75 e
	1.5	1.90 h-k	3.80 abc	2.70 d-i	2.70 d-i	2.78 c
	3	2.50 e-i	4.20 a	3.90 abc	2.30 e-j	3.23 ab
	4.5	2.30 e-j	2.10 f-j	2.40 e-i	2.20 f-j	2.25 d
Terminal	0	1.00 k	1.70 ijk	2.10 f-j	2.80 d-h	1.90 de
	1.5	2.30 e-j	3.00 c-g	3.10 b-f	3.30 a-e	2.93 bc
	3	2.80 d-h	3.60 a-d	3.80 abc	4.00 ab	3.55 a
	4.5	2.20 f-j	2.00 g-j	2.20 f-j	2.40 e-i	2.20 d

Type of buds	IAA mgl^{-1}				Effect of Type of buds
	0	0.2	0.4	0.6	
Lateral	2.00 d	2.98 ab	2.68 abc	2.35 cd	2.50 a
Terminal	2.08 d	2.58 bc	2.80 abc	3.13 a	2.64 a

BA mgl^{-1}	IAA mgl^{-1}				Effect of BA mgl^{-1}
	0	0.2	0.4	0.6	
0	1.15 h	1.75 gh	1.90 fg	2.50 c-f	1.83 d
1.5	2.10 efg	3.40 ab	2.90 bcd	3.00 bc	2.85 b
3	2.65 cde	3.90 a	3.85 a	3.15 bc	3.39 a
4.5	2.25 d-g	2.05 efg	2.30 d-g	2.30 d-g	2.23 c

*Means followed by the same letter within a column do not differ significantly (α=0.05) according to Duncan's Multiple Range Test (Duncan ,1955).

Table (6): The effect of BA concentrations and their interactions with IAA levels on the average length of new shoots (cm) of lateral and terminal buds at multiplication stage. (3 mgl^{-1} of GA_3 were added to all the treatments).

Type of buds	BA mgl^{-1}	IAA mgl^{-1}				Type of buds X BA mgl^{-1}
		0	0.2	0.4	0.6	
Lateral	0	1.20 ij	1.90 ghi	1.60 hij	1.60 hij	1.58 d
	1.5	2.80 a-g	2.60 b-g	3.10 a-d	3.40 ab	2.98 ab
	3	2.90 a-f	2.50 b-h	2.60 b-g	3.00 a-e	2.75 b
	4.5	3.30 abc	2.00 f-i	2.60 b-g	2.70 a-g	2.65 b
Terminal	0	0.80 j	2.00 f-i	1.60 hij	2.20 d-h	1.65 d
	1.5	2.70 a-g	2.40 c-h	2.50 b-h	3.30 abc	2.73 b
	3	3.00 a-e	3.00 a-e	3.30 abc	3.60 a	3.23 a
	4.5	2.20 d-h	2.50 b-h	2.20 d-h	2.10 e-h	2.25 c

Type of buds	IAA mgl^{-1}				Effect of Type of buds
	0	0.2	0.4	0.6	
Lateral	2.55 abc	2.25 bc	2.48 abc	2.68 ab	2.49 a
Terminal	2.18 c	2.48 abc	2.40 abc	2.80 a	2.46 a

BA mgl^{-1}	IAA mgl^{-1}				Effect of BA mgl^{-1}
	0	0.2	0.4	0.6	
0	1.00 f	1.95 de	1.60 e	1.90 de	1.61 c
1.5	2.75 abc	2.50 bcd	2.80 abc	3.35 a	2.85 a
3	2.95 ab	2.75 abc	2.95 ab	3.30 a	2.99 a
4.5	2.75 abc	2.25 cd	2.40 bcd	2.40 bcd	2.45 b

*Means followed by the same letter within a column do not differ significantly (α=0.05) according to Duncan's Multiple Range Test (Duncan ,1955).

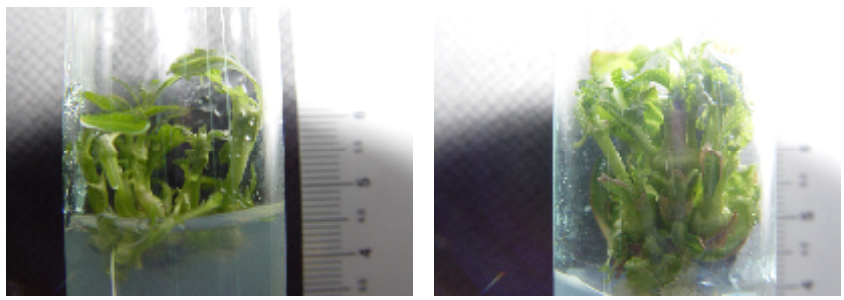


Image (2): Shoots multiplication of *Gardenia jasminoides* on MS medium supplemented with BA+IAA at different concentrations after 4-6 weeks of culture.

ROOTING STAGE: Effect of NAA and MS salt concentrations on *in vitro* rooting of Gardenia shoots. Tables (7 and 8) show the effects of medium salt strength and their interaction with NAA concentrations on average root numbers and lengths /shoot. By increasing NAA concentration from 0 to 4, an increase in the mean number and length of roots/shoot was observed in both full and half strength media and the highest mean number of root /shoot was obtained from half strength MS media containing 4mgl^{-1}NAA (4.00).The average length of root /shoot was significantly affected by NAA and salt concentration of the medium in which by increasing the NAA concentration in both media, the average length of root /shoot increased too. The highest average root length /shoot was obtained in half strength MS medium (4.17 cm) (Table 8). NAA at 4 mgl^{-1} has produced more roots and more root lengths /shoot. No significant effects of media strength was observed in case of root number ,however, in case of root lengths, half strength media has produced more root length /shoot.

Table (7): Effect of medium salt strength and NAA concentrations on the average number of roots/shoot.

Salt strength of MS medium	NAA mgl^{-1}					Effect of salt strength of MS medium
	0	1	2	3	4	
Half strength	0.00 b	2.80 a	3.20 a	3.20 a	4.00 a	2.64 a
Full strength	0.00 b	2.67 a	3.00 a	3.67 a	3.67 a	2.60 a
Effect of NAA mgl^{-1}	0.00 c	2.75 b	3.13 ab	3.38 ab	3.88 a	Overall mean = 2.63

*Means followed by the same letter within a column do not differ significantly (α=0.05) according to Duncan's Multiple Range Test (Duncan ,1955).

Table (8): Effect of medium salt strength and NAA concentrations on the average length of roots (cm).

Salt strength of MS medium	NAA mgl^{-1}					Effect of salt strength of MS medium
	0	1	2	3	4	
Half strength	0.00 e	2.33 bc	2.17 bcd	2.67 b	4.17 a	2.27 a
Full strength	0.00 e	1.24 d	1.38 cd	1.90 bcd	2.50 b	1.40 b
Effect of NAA mgl^{-1}	0.00 c	1.65 b	1.68 b	2.19 b	3.13 a	Overall mean = 1.73

*Means followed by the same letter within a column do not differ significantly (α=0.05) according to Duncan's Multiple Range Test (Duncan ,1955).

Effect of IAA and MS salt concentrations on *in vitro* rooting of Gardenia shoots. Tables (9 and 10) reveal the effects of medium salt strength and their interaction with IAA levels on the average number of roots and their lengths. By increasing IAA concentration from 0-8, a significant increase in the mean number and length of root /shoot was observed .The average number and length of root /shoot (3.88 and 3.13) respectively were obtained in MS medium containing 8 mgl^{-1} IAA. Although the salt strength had no significant effect in the average root number and length /shoot, the highest root number (2.93 roots/shoot) and root length (2.08 cm) was observed in half strength MS medium. As for the interaction between medium salt strength and IAA concentrations, the highest root number /shoot (4.67 roots/shoot) and average root length (3.30 cm) were obtained in half strength MS medium containing 8 mgl^{-1} IAA. These results proved that auxins have a role in rooting process since they promote adventitious roots initiation in the bases of cultured shoots. Root initial cells division depends on both endogenous and exogenous auxins concentration. The physiological effects of auxins are represented in increasing of cell division or converting the matured differentiated cells in shoots bases into merestimatic cells (totipotent cells), so adventitious roots meristem will be formed and its cells will divide to produce adventitious roots (Abdul, 1987 and Saleh, 1991). Endogenous hormones might have a role in promoting shoots to root (Peak *et al*, 1987), until the hormonal balance reached its optimal level to push the roots to grow and develop in the presence of exogenous hormones, since increasing of auxins concentration promotes root formation on shoots bases (George and Shermington, 1984).

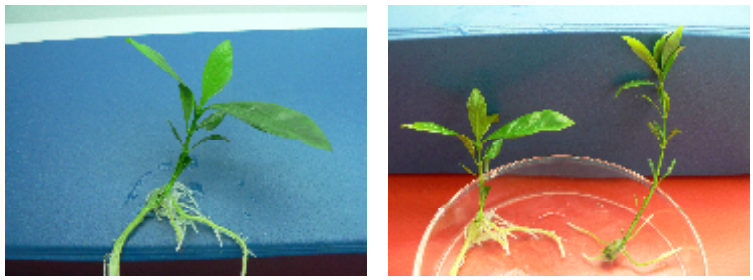
Table (9): Effect of medium salt strength and IAA concentrations on the average number of roots/shoot.

Salt strength of MS medium	IAA mgl^{-1}					Effect of salt strength of MS medium
	0	2	4	6	8	
Half strength	0.00 e	2.33 d	4.00 ab	3.67 abc	4.67 a	2.93 a
Full strength	0.00 e	3.00 bcd	2.40 cd	3.20 bcd	3.40 bcd	2.48 a
Effect of IAA mgl^{-1}	0.00 c	2.75 b	3.00 b	3.38 ab	3.88 a	Overall mean = 2.65

Table (10): Effect of medium salt strength and IAA concentrations on the average length of roots (cm)

Salt strength of MS medium	IAA mgl^{-1}					Effect of salt strength of MS medium
	0	2	4	6	8	
Half strength	0.00 d	1.90 bc	2.10 bc	2.70 ab	3.30 a	2.08 a
Full strength	0.00 d	2.00 bc	1.67 c	2.17 bc	2.83 ab	1.73 a
Effect of IAA mgl^{-1}	0.00 c	1.94 b	1.94 b	2.50 b	3.13 a	Overall mean = 1.95

*Means followed by the same letter within a column do not differ significantly (α=0.05) according to Duncan's Multiple Range Test (Duncan ,1955).



a- b-

Image (3): Root initiation of *Gardenia jasminoides* on MS medium supplemented with a-NAA and b-IAA at different concentrations after 4-6 weeks of culture.

ACCLIMATIZATION STAGE: The successful moving of plantlets from culture tubes to the soil is one of the most important steps in vegetative micropropagation program of any plant species. The results of the present study revealed the ability of plants to depend on themselves and convert to autotrophic. To obtain that, the following steps have been adopted:

1.Washing the plants with tap water after being out of tubes to remove the residues of culture medium which is a goal of microorganisms attacks because of its sugar and agar content. It is preferred to emerge the plants into a fungicide solution (Benlet, $1gl^{-1}$) to protect them from fungal attacks, and then planting them in plastic pots contained a mixture medium (sand and peatmoss, 1: 2) which was a good medium in handling the required humidity to grow the plants well, furthermore, its nutrient elements content. Another fungicide spray was necessary after two weeks to cure any possible new infection. The transplanted plants were irrigated by quarter salts power solution.

2.Covering the plants with light plastic covers to maintain high humidity around the plants and prevent their drought, death and allowing light penetration to the plants to promote enzymes responsible for photosynthesis in order to synthesis food to be converted from heterotrophic to autotrophic.

3.Gradually raising of plastic covers after 2- 3 weeks from plants to ensure plants life and to adopt with natural environmental conditions. In the case of covering for less than two weeks, plants have been drought because of high levels of transpiration after losing the thin layer of water vapor that was directly surrounding the leaves in the suitable environment for leaves, which is usually known as

microenvironment. While in case of covering for more than two weeks, high humidity caused the appearance of fungi on soil surface and plant stems. Following these steps of vegetative micropropagation agrees with what have been found by many researches in fruit plants which were moved to open air field like peaches (Reeves *et al*, 1983), walnut (Mc Granahan *et al*, 1988), chestnut (Preece and Sutter, 1991; Awad, 1995; Trigiano and Gray, 1996 and Ghazal, 1997).It is essential to raise transplanting success rate to the soil to about 100% to get the maximum benefits from this technique. Our results indicated that 95% of transplants were succeeded after transplanting.

Image (4): Plantlets established in pots after 6-8 weeks of transfer.

Image (5): Plant after 8-10 weeks ex vitro.

تأثير التراكيز المختلفة من BA و IAA في الاكثار الدقيق لنبات الكاردينيا *Gardenia jasminoides*

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الخلاصة

نفذت عدة تجارب في مختبر زراعة الانسجة النباتية- كلية الزراعة/ جامعة دهوك للمدة من تشرين الثاني ٢٠٠٥ وحتى تشرين الاول ٢٠٠٦ وذلك لاكثار نبات الكاردينيا *Gardenia jasminoides* خارج الجسم الحي باستعمال اطراف الافرع وعقد مفردة حاوية على براعم ابطية ووسط MS المجهز بتراكيز مختلفة من السايتوكاينينات والاوكسينات. اظهرت النتائج ان لنوع المادة المستعملة في التعقيم تاثير كبير في الحد من نسبة تلوث الاجزاء النباتية المزروعة خارج الجسم الحي, اذ ادى استعمال كلوريد الزئبق ($HgCl_2$) بتركيز ٠.١ % (وزن / حجم) و لمدة ١٠ دقائق كان فعالا في خفض نسبة التلوث الى ادنى حد, وبلغت نسبة البقاء في الاجزاء النباتية المزروعة المعقمة بهذه المادة ٩٩% . في مرحلة النشوء اظهرت النتائج بان اعلى معدل لعدد الافرع والأوراق عند زراعة العقد المفردة على وسط MS المجهز ب(٣ملغم/لتر BA +٠.٩ ملغم/لتر IAA) وأعلى معدل لطول الفرع عند الزراعة في وسط MSالمجهز ب (٤.٥ملغم/لتر BA + ٠.٦ ملغم/لتر IAA). اما بالنسبة لمرحلة التضاعف الخضري، اعطت العقد المفردة المزروعة على وسط MS المجهز ب (١.٥ و٤.٥ملغم/لتر BA + ٠.٦ ملغم/لتر IAA) اعلى معدل لطول الفرع و لعدد الأفرع. وأعلى معدل لعدد الأوراق عند زراعتها على وسط MS

المجهز (٣ ملغم/ لتر BA+ ٠.٢ ملغم/لتر IAA) . بهدف تشجيع الفروع الناتجة عند مرحلة التضاعف الخضري للتجذير يمكن الملاحظة بان المعاملة ٤ ملغم / لتر NAA و٨ ملغم/ لتر IAA أعطوا أعلى نسبة تجذير واعلى قيمة لعدد وطول الجذور. تم وبنجاح اقلمة النباتات الناتجة من الزراعة النسيجية ونقلها الى التربة وبنسبة نجاح بلغت95 % .

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