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Effect of Different Concentrations of Kinetin and NAA 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%). During the culture of shoot tips at initiation stage, a nutrient medium contained (2 mgl^{-1} kinetin+ 0.6 mgl^{-1} NAA) gave the highest values of average number of shoots and leaves and growth length (2.3 shoots/ explant, 2.4 leaves and 1.9 cm respectively).At vegetative multiplication stage, The culture of shoot tips on a nutrient medium containing (3 mgl^{-1} kinetin) gave the highest values of average number of shoots and leaves (2.9 shoots/ explant, 3.6 leaves respectively).As for growth length, the results revealed that the highest value was achieved by MS medium supplemented by 2 mgl^{-1} kinetin + 0.1 mgl^{-1} NAA (3.3 cm). Regarding rooting process, MS medium supplemented by 4 mgl^{-1} NAA gave the highest average number and length of roots (4.0 roots and 2.50 cm respectively).Also, the medium supplemented by 8 mgl^{-1} IAA gave the highest number and length of roots (3.40 roots and 3.50 cm respectively). Plantlets obtained were transferred to pots and acclimatized with 95 % success.

Keywords: Gardenia jasminoides, Micropropagation, Tissue Culture, Plant Growth Regulators.

Introduction

Gardenia jasminoides or common Gardenia is a member of the family Rubiceae and belongs to the genus Gardenia. There are over 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 (Clemson Extension, 1999).White Gardenia blooms are borne from mid spring to early summer, a number of flowers opening over a fairly long season. Gardenias can be used as screens, hedges, borders or ground covers. They may also be used as free-standing specimens or in mass plantings.

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Cultivars of Gardenia jasminoides can be propagated by cuttings or grafting. Cuttings can be taken any time during the year. Propagation could be done by grafting scion from a desired cultivar to a seedling rootstock of Gardenia thunbergia. Rootstock seedlings, however, are difficult to obtain due to problems in seed germination (Bradshaw and Joan, 1998).Micropropagation means the production of a new plant by growing a very small piece of plant tissue in aseptic conditions, usually a test-tube, flask, or bottle, containing a nutrient medium. The medium may be liquid or solidified with agar (Razdan, 2003).Usually, plant growth

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regulators must be supplied in the medium for growth and development of the explants. Plant growth regulators exert dramatic effects at low concentrations. They regulate the initiation and development of shoots and roots on explants and embryos on semisolid or in liquid medium cultures. They stimulate cell division and expansion. 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. Auxins play a role in many developmental processes, including cell elongation and swelling of tissue, apical dominance and adventitious root formation. Cytokinins promote cell division and stimulate initiation and growth of shoots in vitro .They promotes axillary shoot formation by opposing apical dominance regulated by auxins.Gibberellins are less commonly used in plant tissue culture. Of the many gibberellins thus far described, gibberellic acid (GA_3) is the most often used, but it is very heat sensitive. Gibberellins help to stimulate elongation of internodes and have been proved to be necessary for meristem growth for some plant species (Trigiano and Gray, 2000).Wickson and Thiman (1958) used cytokinins in cultured medium of Pisum sativum. Axillary buds started to grow and develop into shoots that could be isolated and rooted separately on certain culture media. According to Murashige (1984) there are more than 3000 plant species which have been vegetative propagated by tissue culture. Jones (1967) was able to put a program for apple propagation by using tissue culture technique. He demonstrated throughout his experiments the effects of cytokinin on promoting shoot growth from cultured shoot apices grown in vitro. Shoot tip culture was also used by Dutcher and Powell (1972) Elliott (1972) and Walkey (1972) who have got intact plants for two varieties of apple by culturing shoot apices of apples taken from small seedlings.Arya and Shekhawat (1986) referred to generation of multiple growths from shoot tips of two cultivars of Zizyphus spina-christi wild (Gola and Seb) cultured on MS medium enriched by 2 mgl^{-1} kinetin. In contrast, Gruselle et al. (1987) noticed that low effectivity of kinetin led to low growth and development of walnut axillary buds. BA and kinetin are the most common cytokinins, whereas, zeatin is less common because of its high costs which limited its use inspite of its high efficiency, however, it is used in some investigations (Trigiano and Gray, 1996).Bashi (1988) pointed out that interaction between kinetin (1, 1.5 and 2 mgl^{-1}) and IBA (0.5, 1.0, 1.5 and 2 mgl^{-1}) formed callus in different degrees. Singh et al. (1994) found that the formula of 1 mgl^{-1} BA, 0.5mgl^{-1}Kinetin and 0.5mgl^{-1} NAA led to the best multiplication of mandarin and lemon shoots. In gardenia, Al-Juboory et al. (1998) found that a comparison of cytokinin activity 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 show 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.

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 (kinetin) 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 3year old *Gardenia jasminoides* grown in the greenhouse of the Department of Horticulture/College of Agriculture at University of Dohuk. 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 the [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 (Tisserat and Zaid, 1983; Mohammed and Omer, 1990 and Olivares *et al*, 1990).

3. Explants Disinfestation: Shoot tips and nodes with axillary buds were removed and disinfested by immersion in the 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. The experiments were conducted with a ten replicates and the explants were placed aseptically in 25x150 mm test tubes containing 15ml of MS medium. Observations were recorded after an interval of four weeks. In the preliminary disinfestations study, the parameters recorded for the assessment of different NaOCl, Ethyl alcohol and that of mercuric chloride were:

1. The number of explants having contamination.
2. The number of healthy explants obtained.

This evaluation was performed on a weekly basis for four consecutive weeks. At the end of the fourth week, the results were compiled, averaged and expressed as a percentage for each treatment.

Culture Initiation Stage: Kinetin Concentrations: The role of kinetin has been tested to reveal its effect on the growth of shoots by adding it at 0, 1, 2 and 3 mgl^{-1} to the culture medium and observing the response of cultured explants by planting ten explants (an explant in each test tube for each concentration). Readings were taken after 4-6 weeks from planting.

The Interaction between Kinetin and NAA: Different concentrations of kinetin

and NAA were tested to reveal their effects on culture initiation when combined together. Kinetin was tested with 0, 1, 2 and 3 mgl^{-1} and NAA with 0, 0.2, 0.4 and 0.6mgl^{-1}. Ten test tubes were used for each treatment under conditions of 16 light hours and 8 darkness hours.

Multiplication Stage: On the basis of stage I results, produced shoots from the treatments were moved to MS medium (multiplication stage medium) from the best treatment. They were incubated in 24±1°C under light conditions of 16 hours and 8 darkness hours. Number and length of shoots were recorded after 6 weeks from planting. Multiplication stage experiments included the following.

Kinetin Concentrations: The effect of kinetin was tested with 0, 1, 2 and 3mgl^{-1} to find out its effect on the number of vegetative growths and their lengths in order to choose the best concentration.

The Interaction between Kinetin and NAA: In order to study the effect of interaction between kinetin and NAA on the number and length of vegetative growth, kinetin was added with 0, 1, 2 and 3 mgl^{-1} and NAA was added with 0.1, 0.2 and 0.3 mgl^{-1}.GA_3 was added to MS medium with 2mgl^{-1} to all the treatments including control treatment.

Rooting Stage: Auxins Levels: The effect of IAA and NAA added to the culture medium on shoots produced at vegetative multiplication stage to root, 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. As far as stage 3 rooting is concerned, features such as number of rooted shoots, root number /shoot and root length (cm) were recorded .This evaluation was performed on a weekly basis for 4-6 consecutive weeks. At the end of six weeks, the results were compiled, averaged and expressed as a percentage or number for each treatment.

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 original power. 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 salts X5 auxin concentrations). Data scored on percentage were subjected to arsine transformation before analysis and then converted back to percentage for presentation. 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 kinetin and NAA concentrations on explant establishment. Tables (1,2and 3) shows the effect of Kinetin concentrations and

their interactions with NAA levels and type of buds on the average number of new shoots, leaves and average length of new shoots. It can be noticed that using high concentrations of kinetin (2 and 3 mgl^{-1}) led to getting the highest responses of number of shoots (1.68 and 1.76 shoots/explant), number of leaves (1.9 and 1.99 leaves/explant) and length of new shoots (1.34 and 1.49 cm) for lateral and terminal buds respectively. These results agree with those published by Singha (1982); Singha *et al*. (1985); Takashi *et al.* (1987); Kyoichi *et al.* (1987) while they were propagating pears and by Druart and Gruselle (1986) while they were propagating plum and by Raheef *et al*. (2000) while they were propagating apples in that adding of cytokinins at culture initiation stage is necessary. Most of the studies affirm the necessity of the presence of cytokinins in the culture medium in concentrations of about 0.2 to $3mgl^{-1}$. Seyhan and Ozzambak (1994) also mentioned that using of cytokinins in $0.5mgl^{-1}$ gave the best bud growth for both olive cultivars (Memecik and Domat) after 35- 40 days from culture. Significant interactions between the type of buds and kinetin levels were observed. Treatment of 2 mgl^{-1} of kinetin with terminal buds gave the highest significant values for the number of new shoots and length of new shoots, whereas in the case of number of leaves/explant, significant value was obtained from the interaction of 3 mgl^{-1} kinetin and terminal buds. It is clear from these tables that terminal buds have produced more new shoots, number of leaves and the lengths of new shoots as compared with those of lateral buds. This may be due to the role of cytokinin in promoting the growth of apical meristem and formation of new shoots through the equilibrium with endogenous auxins that are manufactured by the apical meristem. These results agree with those recorded by Singha *et al*. (1985) and Takashi *et al*. (1987) in pears and with Druart and Gruselle (1986) in plum. Concerning the interaction between kinetin, NAA and types of buds, it is clear that the highest values of number of shoots, number of leaves and the length of new shoots were obtained from the interaction between the high concentrations of both growth regulators for both lateral and terminal buds. The treatment resulted in a significant increase of the average number of new shoots, average number of leaves and average length of new shoots on terminal buds as compared with those of lateral buds. This may be due to cytokinins deficiency in the lateral bud (Stern *et al.* 2004). Experiments with pea plants have shown that axillary buds begin to grow as little as 4 hours after terminal bud has been removed .If cytokinins are applied in appropriate concentration to axillary buds, however, they will begin to grow, even in the presence of terminal buds, there by offsetting apical dominance (Stern *et al.* 2004).In addition to that, axillary buds are commonly initiated somewhat later than the leaves subtending them. Consequently, it is not always clear whether the meristem of the axillary bud is derived directly from apical meristem of the main shoot or whether it originates from partly differentiated tissue of the internodes (Esau, 1965).As mentioned above cytokinins increases cell division more effectively when they interact with auxins. Similar results have been recorded in *Gardenia* by Abdulla *et al*. (2003) where they state that many plantlets were obtained by culturing shoot cuttings of Gardenia in MS nutrient medium containing different concentrations of BA and IAA.

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

Type of buds	Kinetin mgl^{-1}	NAA mgl^{-1}				Type of buds X Kinetin mgl^{-1}
		0	**0.2**	**0.4**	**0.6**	
Lateral	**0**	1.00 e	1.20 de	1.40 b-e	1.50 b-e	1.28 d
	1	1.30 cde	1.20 de	1.40 b-e	1.60 b-e	1.38 cd
	2	1.40 b-e	1.40 b-e	1.60 b-e	1.80 a-d	1.55 abc
	3	1.60 b-e	1.50 b-e	1.90 abc	2.00 ab	1.75 ab
Terminal	**0**	1.00 e	1.40 b-e	1.60 b-e	1.80 a-d	1.45 cd
	1	1.40 b-e	1.30 cde	1.60 b-e	1.80 a-d	1.53 bcd
	2	1.70 bcd	1.40 b-e	1.80 a-d	2.30 a	1.80 a
	3	1.50 b-e	1.70 bcd	1.90 abc	2.00 ab	1.78 ab

Type of buds	NAA mgl^{-1}				Effect of Type of buds
	0	**0.2**	**0.4**	**0.6**	
Lateral	1.33 c	1.40 c	1.58 bc	1.73 ab	1.49 b
Terminal	1.33 c	1.45 c	1.73 ab	1.98 a	1.64 a

Kinetin mgl^{-1}	NAA mgl^{-1}				Effect of Kinetin mgl^{-1}
	0	**0.2**	**0.4**	**0.6**	
0	1.00 f	1.30 def	1.50 cde	1.65 bcde	1.36 b
1	1.35 def	1.25 ef	1.50 cde	1.70 abcd	1.45 b
2	1.55 cde	1.40 de	1.70 abcd	2.05 a	1.68 a
3	1.55 cde	1.60 cde	1.90 abc	2.00 ab	1.76 a

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

type of buds	Kinetin mgl^{-1}	NAA mgl^{-1}				Type of buds X Kinetin mgl^{-1}
		0	**0.2**	**0.4**	**0.6**	
Lateral	**0**	1.00 h	1.10 gh	1.30 fgh	1.30 fgh	1.18 e
	1	1.50 d-h	1.40 e-h	1.50 d-h	1.70 b-f	1.53 d
	2	1.80 b-f	1.60 c-g	1.80 b-f	2.00 a-e	1.80 abc
	3	1.90 a-f	1.70 b-f	1.90 a-f	2.20 abc	1.93 ab
Terminal	**0**	1.00 h	1.40 e-h	1.90 a-f	2.10 a-d	1.60 cd
	1	1.70 b-f	1.60 c-g	1.70 b-f	2.00 a-e	1.75 bcd
	2	1.90 a-f	1.70 b-f	2.00 a-e	2.40 a	2.00 ab
	3	2.00 a-e	1.80 b-f	2.10 a-d	2.30 ab	2.05 a

Type of buds	NAA mgl^{-1}				Effect of Type of buds
	0	**0.2**	**0.4**	**0.6**	
Lateral	1.55 cd	1.45 d	1.63 cd	1.80 bc	1.61 b
Terminal	1.65 cd	1.63 cd	1.93 b	2.20 a	1.85 a

Kinetin mgl^{-1}	NAA mgl^{-1}				Effect of Kinetin mgl^{-1}
	0	**0.2**	**0.4**	**0.6**	
0	1.00 f	1.25 ef	1.60 cde	1.70 cd	1.39 c
1	1.60 cde	1.50 de	1.60 cde	1.85 bcd	1.64 b
2	1.85 bcd	1.65 cd	1.90 abcd	2.20 ab	1.90 a
3	1.95 abc	1.75 cd	2.20 abc	2.25 a	1.99 a

Table (3): The effect of Kinetin concentrations and their interactions with NAA levels on the average length of new shoots (cm) of lateral and terminal buds at

Type of buds	Kinetin mgl^{-1}	NAA mgl^{-1}				Type of buds X Kinetin mgl^{-1}
		0	**0.2**	**0.4**	**0.6**	
Lateral	0	0.60 g	0.70 fg	0.75 efg	1.00 b-g	0.76 c
	1	0.90 c-g	0.90 c-g	1.15 b-g	1.30 b-e	1.06 b
	2	1.00 b-g	0.90 c-g	1.30 b-e	1.40 a-d	1.15 b
	3	1.40 a-d	1.35 a-d	1.60 ab	1.60 ab	1.49 a
Terminal	0	0.60 g	1.00 b-g	1.20 b-f	1.40 a-d	1.05 b
	1	0.80 d-g	1.00 b-g	1.20 b-f	1.60 ab	1.15 b
	2	1.60 ab	1.30 b-e	1.30 b-e	1.90 a	1.53 a
	3	1.45 abc	1.40 a-d	1.50 abc	1.60 ab	1.49 a

Type of buds	NAA mgl^{-1}				Effect of Type of buds
	0	**0.2**	**0.4**	**0.6**	
Lateral	0.98 c	0.96 c	1.20 bc	1.33 b	1.12 b
Terminal	1.11 bc	1.18 bc	1.30 b	1.63 a	1.30 a

Kinetin mgl^{-1}	NAA mgl^{-1}				Effect of Kinetin mgl^{-1}
	0	**0.2**	**0.4**	**0.6**	
0	0.60 g	0.85 fg	0.98 efg	1.20 b-f	0.91 c
1	0.85 fg	0.95 efg	1.18 c-f	1.45 abcd	1.11 b
2	1.30 a-e	1.10 def	1.30 a-e	1.65 a	1.34 a
3	1.43 abcd	1.38 abcd	1.55 abc	1.60 ab	1.49 a

Shape (1): Shoots initiation of *Gardenia jasminoides* on MS medium supplemented with kinetin+NAA at different concentrations after 4-6 weeks of culture.

MULTIPLICATION STAGE: Effect of kinetin and NAA on shoot proliferation. Tables (4, 5 and 6) illustrate the effect of different concentrations of kinetin and their interactions with NAA and types of buds on the average number of shoots, leaves and length of new shoots at multiplication stage. The use of high concentrations of kinetin (2 and 3 mgl^{-1}) gave the highest number of shoots 2.09 and 2.03 shoots/ explant. Concerning the number of leaves and growth length of new shoots, the highest values were recorded for 2 and 1mgl^{-1} kinetin which were estimated at (2.73 leaves/ explant) and (2.6 cm) respectively. The reason behind the increase in the number of shoots is due to cytokinins role in promoting axillary buds growth and ending apical dominance. This agrees with what was found by Arya and Shekhawat (1986) that multi shoots were generated from shoot tips of two cultivars of *Zizyphus spina-christi* wild (Gola and Seb) cultured on MS medium supplemented by 2 mgl^{-1} kinetin .Significant interactions between the types of buds and kinetin levels were noted concerning the characters under study. In general the highest significant values were recorded from treatment of terminal buds with 3 mgl^{-1} kinetin. It is clear from these tables that terminal buds did produce more new shoots and numbers of leaves as compared with those of lateral buds, but no significant differences were recorded regarding the length of new shoots. Regarding the interaction between kinetin ,NAA and types of buds ,it is clear that for average number of new shoots and leaves in lateral buds ,the treatment of 2 mgl^{-1} of kinetin and 0.3 mgl^{-1} of NAA resulted in higher values (2.3 and 2.8 shoots/ explant) as compared with (1 and 1.2 shoots/ explant)of controls. For terminal buds, treatment of 3 mgl^{-1} kinetin and 0 mgl^{-1} NAA resulted in the production of (2.9 and 3.6 shoots/ explant) new shoots as compared with (1 and 1.2 shoots/ explant) new shoots of controls .For average length of new shoots, Table (6) shows that for both lateral and terminal buds ,treatment of 2 mgl^{-1} kinetin and 0.1 mgl^{-1} NAA resulted in the production of higher values (2.9 and 3.3 shoots/ explant)respectively as compared with(1.1 shoots/ explant) of controls. The effect of interaction between cytokinins and auxins in vegetative multiplication and increasing growth lengths can be interpreted by the increase of cytokinins role in the presence of auxins as Mohammed and Al-Younis (1991) reported that movement of cytokinins is generally activated in the presence of auxins, so a larger number of buds will have a chance to grow and start to produce shoots (Tran Thanh, 1981 and Murashige, 1990). These results are in agreement with those reported by Pasqual and Audo, (1989); Huetteman and Preece, (1993); Kahia, (1993); Seyhan and Ozzambak, (1994); Singh *et al*, (1994) and Khairallah, 1997) who emphasized the importance of the interaction between auxins and cytokinins in vegetative multiplication processes. Similar results are reported by Ruginin and Fontanazza,(1981); Rugini ,(1984); Fiorino and Leva ,(1986); Berenguer and Gonzales,(1990) and Rugini ,(1990) in olive propagation category when number of shoots reached to 3 shoots/ explant by using half power MS medium.

Table (4): The effect of Kinetin concentrations and their interactions with NAA levels on the average number of new shoots of lateral and terminal buds at multiplication stage. (2 mgl^{-1} of GA_3 were added to all the treatments).

Type of buds	Kinetin mgl^{-1}	NAA mgl^{-1}				Type of buds X Kinetin mgl^{-1}
		0	0.1	0.2	0.3	
Lateral	0	1.00 k	1.40 h-k	1.30 ijk	1.20 jk	1.23 d
	1	2.00 c-h	1.60 f-k	1.50 g-k	1.40 h-k	1.63 c
	2	2.00 c-h	2.00 c-h	2.10 c-g	2.30 a-e	2.10 b
	3	2.20 b-f	1.30 ijk	1.40 h-k	1.50 g-k	1.60 c
Terminal	0	1.00 k	1.50 g-k	1.40 h-k	130 ijk	1.30 d
	1	1.90 c-i	2.20 b-f	1.70 e-j	1.50 g-k	1.83 bc
	2	2.50 abc	2.40 a-d	1.70 e-j	1.70 e-j	2.07 b
	3	2.90 a	2.80 ab	2.30 a-e	1.80 d-j	2.45 a

Type of buds	NAA mgl^{-1}				Effect of Type of buds
	0	0.1	0.2	0.3	
Lateral	1.80 bc	1.58 c	1.58 c	1.60 c	1.64 b
Terminal	2.08 ab	2.23 a	1.78 c	1.58 c	1.91 a

Kinetin mgl^{-1}	NAA mgl^{-1}				Effect of Kinetin mgl^{-1}
	0	0.1	0.2	0.3	
0	1.00 f	1.45 de	1.35 ef	1.25 ef	1.26 c
1	1.95 bc	1.90 bcd	1.60 cde	1.45 de	1.73 b
2	2.25 ab	2.20 ab	1.90 bcd	2.00 bc	2.09 a
3	2.55 a	2.05 bc	1.85 bcd	1.65 cde	2.03 a

Table (5): The effect of Kinetin concentrations and their interactions with NAA levels on the average number of leaves of lateral and terminal buds at multiplication stage. (2 mgl^{-1} of GA_3 were added to all the treatments).

Type of buds	Kinetin mgl^{-1}	NAA mgl^{-1}				Type of buds X Kinetin mgl^{-1}
		0	0.1	0.2	0.3	
Lateral	0	1.20 j	1.40 ij	1.60 hij	1.80 f-j	1.50 e
	1	2.40 c-h	1.70 g-j	1.90 e-j	2.00 d-j	2.00 d
	2	2.60 b-g	2.60 b-g	2.30 c-i	2.80 a-e	2.58 bc
	3	2.20 c-i	2.00 d-j	2.60 b-g	2.30 c-i	2.28cd
Terminal	0	1.20 j	1.40 ij	1.70 g-j	2.00 d-j	1.58 e
	1	2.70 b-f	2.90 a-d	2.20 c-i	1.70 g-j	2.38 cd
	2	3.00 abc	3.40 ab	2.80 a-e	2.30 c-i	2.88 ab
	3	3.60 a	3.30 ab	2.80 a-e	2.40 c-h	3.03 a

Type of buds	NAA mgl^{-1}				Effect of Type of buds
	0	0.1	0.2	0.3	
Lateral	2.10 bc	1.93 c	2.10 bc	2.23 bc	2.09 b
Terminal	2.63 a	2.75 a	2.38 ab	2.10 bc	2.46 a

Kinetin mgl^{-1}	NAA mgl^{-1}				Effect of Kinetin mgl^{-1}
	0	**0.1**	**0.2**	**0.3**	
0	1.20 g	1.40 fg	1.65 efg	1.90 def	1.54 c
1	2.55 abc	2.30 bcd	2.05 cde	1.85 def	2.19 b
2	2.80 ab	3.00 a	2.55 abc	2.55 abc	2.73 a
3	2.90 ab	2.65 abc	2.70 ab	2.35 bcd	2.65 a

Table (6): The effect of Kinetin concentrations and their interactions with NAA levels on the average length of new shoots (cm) of lateral and terminal buds at multiplication stage. (2 mgl^{-1} of GA_3 were added to all the treatments).

Type of buds	Kinetin mgl^{-1}	NAA mgl^{-1}				Type of buds X Kinetin mgl^{-1}
		0	**0.1**	**0.2**	**0.3**	
Lateral	**0**	1.10 j	1.70 g-j	1.40 ij	1.40 ij	1.40 e
	1	2.30 b-h	2.05 c-i	2.10 c-i	2.40 b-g	2.21 bc
	2	2.50 a-g	2.90 abc	2.70 a-e	2.60 a-f	2.68 a
	3	1.90 e-j	1.80 f-j	1.70 g-j	1.90 e-j	1.83 d
Terminal	**0**	1.10 j	1.40 ij	1.70 g-j	1.50 hij	1.43 e
	1	2.00 d-i	2.00 d-i	1.90 e-j	1.70 g-j	1.90 cd
	2	2.40 b-g	3.30 a	2.60 a-f	1.80 f-j	2.53 ab
	3	2.60 a-f	3.10 ab	2.80 a-d	1.90 e-j	2.60 a

Type of buds	NAA mgl^{-1}				Effect of Type of buds
	0	**0.1**	**0.2**	**0.3**	
Lateral	1.95 bc	2.11 abc	1.98 bc	2.08 abc	2.03 a
Terminal	2.03 bc	2.45 a	2.25 ab	1.73 c	2.11 a

Kinetin mgl^{-1}	NAA mgl^{-1}				Effect of Kinetin mgl^{-1}
	0	**0.1**	**0.2**	**0.3**	
0	1.10 f	1.55 def	1.55 def	1.45 ef	2.21 b
1	2.15 bc	2.03 cd	2.00 cde	2.05 cd	2.60 a
2	2.45 bc	3.10 a	2.65 ab	2.20 bc	2.06 b
3	2.25 bc	2.45 bc	2.25 bc	1.90 cde	1.41 c

Shape (2): Shoots multiplication of *Gardenia jasminoides* on MS medium supplemented with kinetin+NAA 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) shows the effect of medium salt strength and their interactions with NAA levels on the average number of roots and their average length respectively. Results in Table (7) indicate that salt strength in MS medium had no significant effect on the average number of roots /shoot whereas it had a significant effect on the length of roots / shoot. Half strength MS medium gave significantly higher root length (1.97 cm) (Table 8).

The length and number of roots /shoot were significantly affected by the concentration of NAA and levels of macro and micro salts of MS medium .The highest root number / shoot (4.67roots/shoot)was obtained in response to the level of 4 mgl^{-1} NAA in half strength macro and micro MS salt (Table 9).As for average length of root/shoot, the best results (3.50 cm) were obtained when high NAA level (4 mgl^{-1})and half strength MS macro and micro salt were combined in culture medium (Table 10).

These results are similar to those obtained by Vengadesan *et al*. (2002),Bhatt and Dnar (2004) and Ozel and Arslan (2006),who observed that reducing the levels of MS salt in the medium to half increased rooting of many tree species. It could be noticed that both salt strength have produced more roots and more root lengths in the presence of higher concentration of NAA (4 mgl^{-1}). These results are similar to those reported by Snir, (1982); Chalupa, (1981) and Penuela *et al*, (1987) who confirmed the importance of the auxin NAA in the rooting process.

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 strengh of MS medim
	0	**1**	**2**	**3**	**4**	
Half strength	0.00 c	3.33 b	3.00 b	3.33 b	4.67 a	2.87 a
Full strength	0.00 c	2.80 b	3.20 b	3.20 b	4.00 ab	2.64 a
Effect of NAA mgl^{-1}	0.00 c	3.00 b	3.13 b	3.25 b	4.25 a	Overall mean = 2.73

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 d	2.00 bc	1.83 bc	2.50 b	3.50 a	1.97 a
Full strength	0.00 d	1.24 c	1.38 c	1.90 bc	2.50 b	1.40 b
Effect of NAA mgl^{-1}	0.00 d	1.53 c	1.55 c	2.13 b	2.88 a	Overall mean = 1.62

Effect of IAA and MS salt concentrations on *in vitro* rooting of Gardenia shoots. Tables (9 and10) illustrate the effect of salt strength of medium and their interactions with IAA levels on the average number of roots and their lengths respectively .With regard to the effects of salt strength in MS medium and their interaction with IAA level on average number and length of roots

/shoot, the results in Table (9) and (10) show that salt strength in MS medium had no significant effects on average number and length of root. The results show that the highest average number of roots and length /shoot occurred in medium supplemented with 8mgl^{-1}IAA. As for interaction between salt strength medium and IAA concentration, the highest average number of roots /shoot (4.33) was obtained on half strength MS salt medium supplemented with 8 mgl^{-1}IAA whereas the highest root length/shoot (3.50 cm) was obtained on full strength MS salt medium supplemented with 8 mgl^{-1} IAA. The concentrations of 6 and 8 mgl^{-1} IAA was determined to be the best for both rooting and root growth .The fact that no rooting occurred in a medium without IAA regardless of the salt levels in MS medium indicate that IAA was essential for root development (Tables 9 and 10).

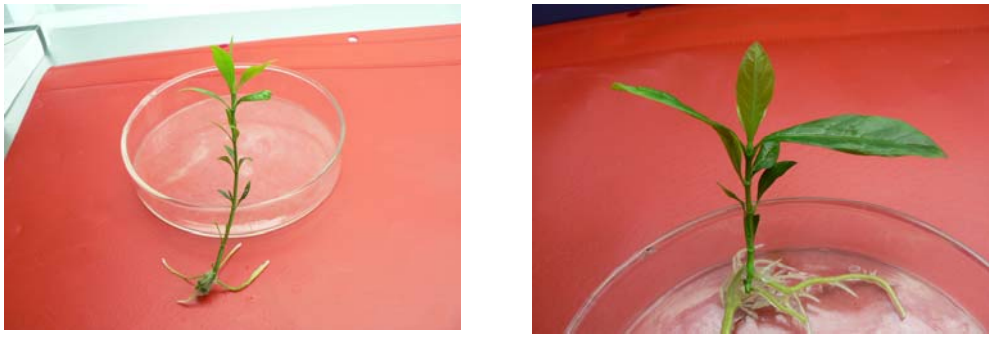
Table (9): Effect of medium salt strength and IAA concentrations on the average number of roots/shoot.

Salt strength of MS medium	IAA mgl^{-1}					Effect of salt strength of MS medium
	0	2	4	6	8	
Half strength	0.00 c	3.33 ab	2.67 b	3.67 ab	4.33 a	2.80 a
Full strength	0.00 c	3.00 ab	2.40 b	3.20 ab	3.40 ab	2.48 a
Effect of IAA mgl^{-1}	0.00 c	3.13 ab	2.50 b	3.38 a	3.75 a	Overall mean = 2.60

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 abc	3.30 a	2.08 a
Full strength	0.00 d	1.83 c	1.83 c	2.83 ab	3.50 a	2.00 a
Effect of IAA mgl^{-1}	0.00 d	1.88 c	2.00 c	2.75 b	3.38 a	Overall mean = 2.05

*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-

Shape (3): Root initiation of *Gardenia jasminoides* on MS medium supplemented with a-IAA and b-NAA 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
4. 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 the case of covering for more that two weeks, high humidity caused the appearance of fungi on soil surface and plant stems. Following these steps of vegetative micropropagation agrees with what has been found by many researches in fruit plants which were moved to open air field like apples (Snir and Erez, 1980), peaches (Reeves *et al*, 1983), walnut (Mc Granahan *et al*, 1988), chestnut (Marie chevre *et al*, 1983; Yang *et al*, 1982 and 1983; Preece and Sutter, 1991; Hameed, 1994; Awad, 1995; Trigiano and Gray, 1996; Khairallah, 1997 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

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

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

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تأثير تراكيز مختلفة من الكاينيتين و NAA على الاكثار الدقيق لنبات الكاردينيا Gardenia jasminoides

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الخلاصه

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(٢,٣٠ فرع/جزء نباتي) وعدد الاوراق(٢,٤٠ ورقة) وطول الفروع (١,٩٠سم) عند زراعة اطراف الافرع في وسط** MS **المجهز ب(٢ملغم/لتر كاينيتين + ٠,٦ ملغم/لتر** NAA**). وفي مرحلة التضاعف الخضري, فقد اعطت اطراف الافرع اعلى معدل لعدد الفروع(٢,٩٠ فرع/جزء نباتي) واعلى معدل لعدد الاوراق(٣,٦٠ ورقة) عند زراعتها على وسط** MS **المجهز(٣ملغم/لتر كاينيتين) وكان اعلى معدل لطول الفرع (٣,٣٠سم) عند الزراعة على وسط** MS **مجهز ب(٣ملغم/لتر كاينيتين + ٠,١ ملغم/لتر** NAA**) .فيما يتعلق بعملية التجذير, الفروع تم زراعتها على وسط** MS **خالي من**

MSالمجهز بـ٤ ملغم / لتر NAA اعطى
اعلى معدل لعدد وطول الجذور على التوالي (٤ جذور و ٢, ٥٠ سم) والوسط المجهز بـ٨ ملغم / لتر IAA اعطى اعلى معدل لعدد وطول الجذور على التوالي (٣, ٤٠ جذور و ٣, ٥٠ سم). كما امكن وبنجاح اقلمة نباتات الناتجة من الزراعة النسيجية ونقلها الى التربة و بلغت نسبة النجاح ٩٥٪.

كاریگەری چڕە جیاوازەکانی سایتۆکاینین(کاینتین) و ئۆکسین لەسەر زۆرکردنی ڕووەکی کاردینیا لە تاقیگەدا .

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پوختــــە

زیدەکرنا کەسکاتیا ڕووەکا گاردینیا بریکا بکارئینا تەکنیکین چاندنا تشیوی *Gardenia jasminoides*ئەڤ ڤەکولینا دلابورا چاندنا تشیویی یا ڕووەکی ل کولیژا چاندنی-زانکویا دهوك بو ماوی 11-12 هەیڤا هاتیەکرن. ڤەکولین پیکهاتیە ژ تەکنیکین چاندنا شانین ڕووەکی لزیدەکرنا کەسکاتیا ڕووەکی گادینیا *Gardenia jasminoides* بریکا چاندنا گرین برخیڤە ئەوین هاتینە ژی ڤەکرن ژ دەرڤەی لەش ڕووەکی زیندی دمیدیایەکا خوارنی MS کو تیراتین جوار جور لسەر هاتینە زیدەکرن ژ ریکەرین گەشەکرنی.گەلەك تاقیکرن هاتینە کرن مینا هەلبژارتنا ریکین نمونەی بو تاقیکرنا پارچین ڕووەکی وهەلبژارتنا ژیدەرین پارچین ڕووەکی ئەوین کیر ب چاندنی دهین, سەرەلایی ڤەکولینا کارتیکرنا ریکخەرین ڤەژاندنی (گەشەکرن) ب تیراتین جوار جور دقوناغین چی بونا شینکاتیا وزیدەبونا کەسکی ورە وریشالا. کاودانین کیرهاتی بن دەشیشانکری بو سەرئیخستنا کریارا ڤەگوهاستنا ڕووەکین شانیدا بو ئاخی هاتنەکرن.ئە نجاما دیارکر کو جوداهیا جوری کەرەستی تاخیرلکرتی کارتیکرن دکتیکرنا پیسبونا شین بوین درووەکا گاردینیا ئاشکرابو کو بکارئینانا ($HgCl_2$) بتیراتیا 0.1% بودەخولەکا جالاکبوی دنە ئاشکرابونا پیسبونی وبلندترین ریژامانی کو ٩٩٪ بو بدەست ڤەهات ,وبومەرەما بدەستڤەئینانا باشترین گەشەکرنی وباشترین سەرەیی,ئە نجاما ئاشکراکر دتاقیکرنائیکی کو چاندنا عوقەدا لگرتین برەخیفەدنیڤیدا ئەوین کو پیکهاتین ژ(٢ ملغم/لیتر kinetin +٠,٦ملغم/لیترNAA) کوبلندترین تیکرا بو هژمارا شینبونا وهژمارابەلکاودریژاهیین گەشەکرنا کو (٢,٣ چقا / پارچەکارووەکی) و(٢,٤) و(١,٩) لدویڤ ئیکرا ب دەستڤەهات. بەلێ دقوناغا کەسکاتی کو چاندنا چقین دقوناغاگەشەکرنیدمیدیات MSێ بەرهەڤکری (٢ملغم/لتر Kinetin+ ٠,٣ ملغم/لتر NAA+ 2ملغم/لتر GA3) بلندترین تیکرا بو هژمارا چقا وهژمار بەلگان دەستڤەهات کو(٢,٣) بکڤە نە نجاما دیارکر بلندترین تیکرا دمیدیاتی بەرهەڤکری ب (٣ملغم/لتر Kinetinبدەستڤەهات. بەلی کریارا پەیدابونا رهو وریشالا ب چاندنا چقا دمیدیاین MSڤالە ژسایتوکاینینات دگەل هەبونا تیراتین بلند ژئوکسینات کو نە نجاما دیارکر کو میدیایی بەرهەڤکری ژ(٤ ملغم/لتر NAA) بلندترین تیکرا ژهژمارا رهەو ریشالا ودریژاهیا رهەو ریشالا کو (٤) و(٢,٥٠)سم لدویڤ ئیکرا ب دەستڤەهات بەلی سەبارت میدیایی بەرهەڤکری ژ(٨ ملغم/لتر IAA) کو دیسان بلندترین تیکرا بو هژمارا رهەوریشالا کو ب (٣,٤) ودریژاهیا رهە وریشالا ب (٣,٥سم) بدەست خوڤەئینان.و (اقلمە) کرنا ڕووە کین بە رهەم هاتی ژ چاندنا شانا ب سەرکەفتیانە هاتە نە نجامدان و هاتنە ڤەگوهاستن بو ناخی و ریژا سەرگەفتنی گەهەشتە ٩٥ ٪ .

وەرگیراوە لە ٢٠٠٨/٩/١٧ دا , و پەسەند کرا لە ٢٠٠٩/٣/٤ دا

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