MICROPROPAGATION OF GARDENIA *Gardenia jasminoides* BY USING SINGLE NODES

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ABSTRACT

The present study was carried out to study the micropropagation of Gardenia (*Gardenia jasminoides*) by using single nodes excised from soft cuttings using half strength MS salts, $30gl^{-1}$ sucrose, $7gl^{-1}$ Agar and different concentrations of plant growth regulators in culture medium. Results of the experiment at initiation stage revealed that the culture of single nodes of Gardenia on a medium containing $5mgl^{-1}$BA gave the highest number of shoots (2.2 shoots/explant). Concerning the interaction, a nutrient medium containing ($2\ mgl^{-1}$ BA+ $0.4\ mgl^{-1}$ IAA) gave the highest values of average number of shoots and leaves and length of new shoots (1.6 shoots/explant, 2 leaves/explant, 1.6cm respectively). At vegetative multiplication stage, the results showed that the medium supplemented by $2\ mgl^{-1}$ BA gave the highest values of average number of shoots and leaves and length of new shoots (2.20 shoots/explant, 3.20 leaves/explant, 1.80cm respectively), whereas the medium supplemented by $0.3\ mgl^{-1}$ IAA gave the highest number of shoots and length of new shoots. In order to promote the shoots produced at vegetative multiplication stage to root, it was noticed that the treatment of $8mgl^{-1}$NAA gave the highest percentage of rooting (90%) and the medium supplemented by $12mgl^{-1}$ IAA gave (100%) rooting. 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. Gardenias can be used as screens, hedges, borders or ground covers. They may also be used as free-standing specimens or in mass plantings. Cultivars of *Gardenia jasminoides* can be propagated by cuttings or grafting. Cuttings can be taken in 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, they are difficult to obtain due to problems in seed germination. Plant tissue culture is the growth of plant organs or tissues in aseptic culture where the environment as well as the nutrient and hormone levels for growth are tightly controlled (Razdan, 2003).

Pontikis and Sapoutzaki(1984) were able to get shoots grown on terminal buds of troyer citrange seedlings grown on MS medium enriched by $0.5\ mgl^{-1}$BA, $0.1mgl^{-1}$ IBA and $0.1\ mgl^{-1}$ GA_3. Pasqual and Audo (1989) promoted vegetative multiplication of tri-foliate orange shoots by using MS medium supplemented by $2\ mgl^{-1}$ BA and $1mgl^{-1}$ NAA. Kyoichi *et al*. (1987) found the best response of shoot tip of pear cultivars (Bartlett, Lelectierr, Silver Bell and Lafrance) when cultured in

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MS half strength medium with 8.88-4.4 *M*m of BA. Werner and Boe (1980) found that MS medium with half salts strength and 0.5mgl^{-1} BA was the optimum medium in M7 apple rootstocks multiplication and they could isolate 13 shoots from one shoot. Fiorino and Leva (1986) Berenguer and Gonzales (1990) and Rugini (1990) had emphasized that number of shoots reached to 3 shoots/explant by using half strength MS medium supplemented with zeatin, GA_3 and IBA in olive propagation. Mante and Tepper (1983) and Hatamla and Al-Khazaela (1990) found that the growth regulator BA with concentration of 1-4 mgl^{-1} is the best concentration in multiplication stage, While Hamad (1996) and Mendes *et al*. (1996) noted that concentration of 4.5,5 mgl^{-1} of BA is the best in multiplication to propagation of *Musa tektiles* in MS nutrient media. Moretti *et al*. (1991) obtained a high percent rooting when pear shoots cultivar (Kaiser) cultured on MS media with half strength supplemented with IAA at 0.49 *M*m. Bader *et al*. (2000) found that perfect rooting (100%) can be obtained at growth shoot culture with pear stock (*Pyrus calleryana*) in multiplication stage on MS medium half strength with 6.66 *M*m NAA which gave 2.7 root/shoot. Abdullah *et al.* (2003) stated that many plantlets were obtained by culturing shoot cuttings of Gardenia in MS nutrient media with 30gl^{-1} sucrose, 7 gl^{-1} agar and different concentrations of BA and IAA .The best combination was 1mgl^{-1} BA with 0.5mgl^{-1} IAA. This treatment gave the best shoot growth suitable for rooting in primary and secondary culture by reculturing the rootstock cutting every 6 weeks and for many times. The objectives of this study were to evaluate the affects of explant type, MS salt strength and different type and concentration of CKs and auxins on in vitro propagation.

MATERIALS AND METHODS

This experiment was carried out at the laboratory of plant tissue culture of Horticulture Department, College of Agriculture, University of Dohuk between June, 2006 and January, 2007.

Plant Materials [Source of Explants and Explant Preparation]: Shoots of *Gardenia jasminoides* 15cm in length were collected from plants 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. After leaves removal, selected shoots were washed under tap water for 1 hour, 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 single nodes with axillary bud. To decrease tissues browning, shoot were placed in cold antioxidant solution containing (150 mgl^{-1}) citric acid and (100 mgl^{-1}) ascorbic acid for 20 minutes followed by 5 minute rinses in sterilized distilled water (Mohammed and Omer,1990 and Olivares *et al*, 1990). The shoots were disinfect by immersion in the solutions of Mercuric Chloride ($HgCl_2$), (0.1%) w/v for 10 minutes. The disinfect tissues [explants] were rinsed 5 times with sterilized distilled water, and the ends of explants exposed to sterile solution were trimmed. The nutrient media contained half inorganic and organic constituents according to (Murashige and Skoog, 1962). Then 30 gl^{-1} of sucrose, 100 mgl^{-1} of myo-inositol, and different

vitamins in addition to several studied growth regulators were added according to the purpose of the experiment and further than containing 7 gl^{-1} agar.

In initiation stage: Different concentrations of BA and IAA were tested to find out their effect on culture initiation both alone and when combined together. BA was used at 0, 2, 3.5 and 5 mgl^{-1} and IAA at 0, 0.2, 0.4 and 0.6mgl^{-1} alone or in combination. 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 1000 lux and 8 darkness hours. The results were recorded after 4-6 weeks from planting.

In multiplication stage: BA was tested at concentrations of 0, 2, 3.5 and 5 mgl^{-1} and IAA at 0, 0.1, 0.2 and 0.3mgl^{-1} alone or in combination. GA_3 was added to MS medium with 3mgl^{-1} to all the treatments including control treatment.

In rooting stage: when the explants reached 2-4cm in length, they were transferred into half strength MS medium containing different concentration of IAA and NAA (one shoot for each test tube and ten test tubes for each treatment). The effect of IAA and NAA added to the culture medium on shoots rooting was studied by carrying out several separate experiments by adding NAA with (0,2, 4, 6 and 8) mgl^{-1} and IAA with (0,3,6,9 and 12) mgl^{-1} ,all these treatments were examined in half strength salt. Number of rooting shoots, root number /shoot and root length (cm) were recorded and 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.

In 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 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 designed as Randomized Complete Block design (RCBD).Ten test tubes were used for each treatment. Data scored on percentage were subjected to arcsine transformation before analysis and then converted back to percentage for presentation. Significant differences among mean values were separated using Duncan multiple range tests at $P \leq 0.05$ (Duncan, 1955).

RESULTS AND DISCUSSION

Initiation Stage: Figure (1) shows that the effect of different concentrations of IAA, BA and their interaction on response percent of the single nodes excised from the soft cuttings of Gardenia cultured on half strength MS salts. Control treatment gave the lowest response percent (60%), whereas the highest concentrations of BA (3.5, 5 mgl^{-1}) gave the highest response percent (100%) which was superior upon

the other concentrations. Concerning the interaction between BA and IAA concentrations the figure shows that the treatment of 2 mgl^{-1} of BA + 0.2,0.4 mgl^{-1} of IAA ,3.5 mgl^{-1} BA + 0,0.2 mgl^{-1} of IAA and 5 mgl^{-1} of BA + 0,0.2 mgl^{-1} of IAA gave the highest response (100%).

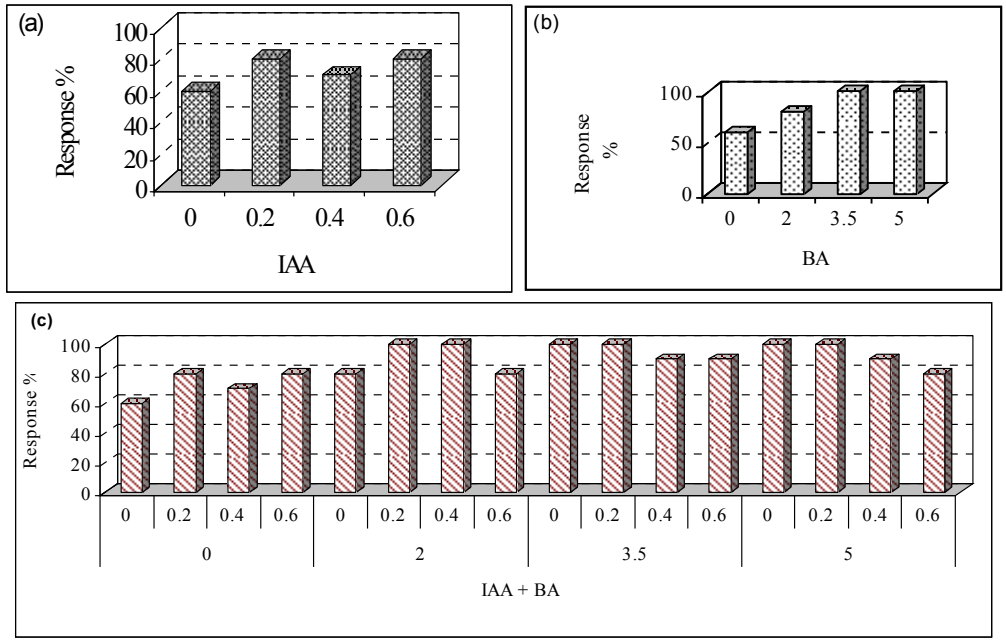


Figure (1): The effects of different concentrations of IAA (a), BA(b) and their interaction (c) on the percentages of response at initiation stage.

Table (1) shows the effect of different concentrations of BA and IAA and their interactions on the average number of shoots, average number of leaves and length of new shoots at initiation stage. Using high concentrations of BA (5 mgl^{-1}) led to obtain the highest number of shoots (2.2 shoots/explant). This may be attributed to cytokimins deficiency in lateral bud as compared with apical bud (Stern *et al*, 2004). whereas concerning number of leaves and shoot length, the lowest concentration of BA (2 mgl^{-1}) recorded the highest values (1.8 leaves/explant and 1.4 cm respectively). These results are in agreement with those found by Kyoichi *et al*. (1987).Whereas the effect of IAA did not differ significantly from the other treatments concerning number of shoots and leaves, but concerning the shoot length, the concentration of IAA (0.4 mgl^{-1})gave the highest values(2cm) as compared with the other concentrations (0.2,0.6 mgl^{-1})IAA. Concerning the interaction between BA and IAA concentrations, it is clear that the treatment of 2mgl^{-1}BA+ 0.4 mgl^{-1} IAA and 5mgl^{-1}BA+ 0.6 mgl^{-1} IAA gave the highest values of number of shoots (1.6 shoots/ explant). The treatment of 2 mgl^{-1} BA + 0.4 mgl^{-1} IAA gave the highest value of number of leaves (2 leaves/explant) in which significantly differed from the control treatment. However control treatment gave the lowest response in which significantly differed with the concentrations used. These result are in agreement with what had been found by Pontikis and Spoutzaki (1984) and Pasqual and Audo (1989) that using of cytokinins and auxin in this

category is very important and the role of cytokinins at this stage is to help the meristems grow and form a vegetative shoot by balancing with auxins auto produced by the meristem.

Table(1): The effect of BA, IAA concentrations and their interactions on the average number of new shoots, number of leaves and length of new shoots at initiation stage.

Treatment mg l^{-1}	Average No. of Branches	Average No. of Leaves	Length of New Shoots (Cm)
BA			
2	1.80 ab	1.80 ab	1.40 ab
3.5	1.80 ab	1.60 ab	1.00 b
5	2.20 a	1.00 ab	1.20 ab
IAA			
0.2	1.40 ab	1.00 ab	1.00 b
0.4	1.80 ab	1.00 ab	2.00 a
0.6	1.80 ab	1.00 ab	1.20 ab
BA + IAA			
2+0.2	1.20 b	1.40 ab	1.40 ab
2+0.4	1.60 ab	2.00 a	1.60 ab
2+0.6	1.40 ab	1.40 ab	1.40 ab
3.5+0.2	1.20 b	1.60 ab	1.20 ab
3.5+0.4	1.20 b	1.20 ab	1.60 ab
3.5+0.6	1.40 ab	1.20 ab	1.20 ab
5+0.2	1.40 ab	1.00 ab	1.40 ab
5+0.4	1.40 ab	0.80 ab	1.40 ab
5+0.6	1.60 ab	1.40 ab	1.60 ab
Control	1.00 b	0.60 b	0.78 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).

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

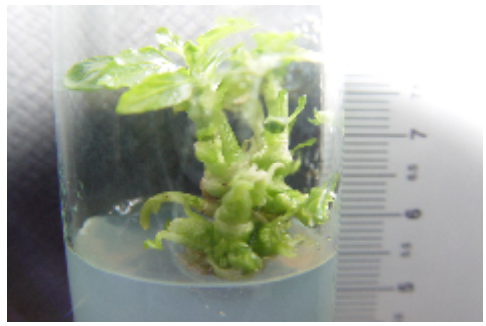
Multiplication Stage: Table (2) reveals the effect of different concentrations of BA and IAA and their interactions on the average number of shoots, average number of leaves and length of new shoots at multiplication stage. That the treatment of 2mgl^{-1} BA gave the highest rate of shoot number and leaves and length of new shoots (2.2

shoots/ explant) and (3.2 leaves/explant) and (1.8 cm) respectively, in which was significantly superior upon other concentrations. This may be due to BA as the most effective cytokinin in cell division as compared with the other cytokinins (Gruselle *et al.*, 1987). Whereas, the effect of IAA did not differ significantly from the other treatment concerning number of shoot, while in case of leaves number the concentration of IAA 0.2mgl^{-1} gave the highest value (2.6 leaves/explant). Concerning the length of new shoots the treatment 0.3mgl^{-1}IAA (3.2cm) which was significantly superior upon other concentrations. This may be due to the effect of auxins in cell wall enlargement (Abdul, 1987). Concerning the interaction treatments reveal that the treatment of 2 mgl^{-1} BA + 0.2 mgl^{-1} IAA gave highest value of the number of shoots and leaves number (2 shoots/explant) and (2.6 leaves/explant) respectively. While concerning the length of new shoots, the treatment of 5mgl^{-1} BA+ 0.3 mgl^{-1} IAA gave the highest value (2.8 cm) These results are in agreement with what have been found by Hatamla and Al-Khazaela (1990) Hamad (1996) and Mendes *et al*.(1996).

Table (2): The effect of BA, IAA concentrations and their interactions on the average number of new shoots, number of leaves and length of new shoots at multiplication stage. (3 mgl^{-1} of GA_3 were added to all the treatments).

Treatment mg l^{-1}	Average No. of Branches	Average No. of Leaves	Length of New Shoots (Cm)
BA+ GA_3			
2+3	2.20 a	3.20 a	1.80 bcd
3.5+3	1.40 abc	2.00 ab	1.60 cd
5+3	1.60 abc	2.60 ab	1.80 bcd
IAA+ GA_3			
0.1+3	1.40 abc	1.40 b	2.00 bcd
0.2+3	1.40 abc	2.60 ab	2.20 abc
0.3+3	1.60 abc	2.20 ab	3.20 a
BA+ IAA+ GA_3			
2+0.1+3	1.60 abc	2.00 ab	2.20 abc
2+0.2+3	2.00 ab	2.60 ab	1.60 cd
2+0.3+3	1.60 abc	2.00 ab	1.80 bcd
3.5+0.1+3	1.40 abc	1.00 b	1.00 d
3.5+0.2+3	1.40 abc	1.80 ab	2.40 abc
3.5+0.3+3	1.00 c	1.40 b	1.60 cd
5+0.1+3	1.20 bc	2.20 ab	1.80 bcd
5+0.2+3	1.00 c	1.80 ab	2.40 abc
5+0.3+3	2.00 ab	2.60 ab	2.80 ab
Control+3	1.00 c	1.00 b	0.94 d

- 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).



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

Rooting Stage: Figure (2) shows the effect of NAA on the rooting percentage of Gardenia shoots cultured on half strength MS medium. It can be noticed that 8mgl^{-1} NAA gave the highest response percent (90%) as compared with the other concentrations. Endogenous hormones might have a role in promoting plants 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). These results are in agreement with those found by Bader *et al*. (2000).

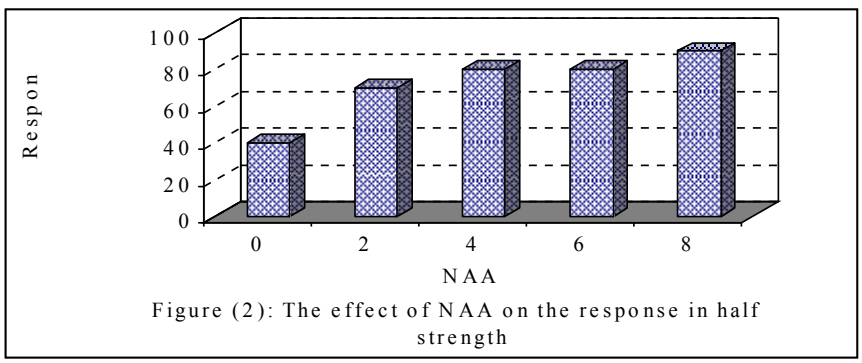


Figure (2): The effect of NAA on the response in half strength

Table (3) shows the effect of NAA concentrations on the average root numbers and lengths /shoots. It is obvious that in case of roots number, there were no significant differences among all concentration except the control treatment. The same table reveal that the high concentrations of NAA (8 mgl^{-1}) produced more root lengths /shoot (2.5) and was significantly superior upon the most other concentrations. These results proved that auxins have a role in rooting process since they promote adventitious roots initiation in the bases of cultured shoots (Abdul, 1987 and Saleh, 1990). These results are in agreement with those found 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. Decreasing the level of salts in the medium means decreasing the level of nitrogen in the medium to half or quantes, this will result in decreasing nitrogen level in the shoots which may result in increasing the percentage of carbohydrates to nitrogen level and this may result in increasing the percentage of root primordial and roots number (Gawel, 1990).

Table (3): Effect of NAA on number and roots length on shoots planted in half strength MS media.

Treatment mg l^{-1} (NAA)	Average No. of Roots	Average Length of Roots
0	0.40 b	0.30 c
2	2.80 a	1.24 b
4	3.20 a	1.38 b
6	3.20 a	1.90 ab
8	4.00 a	2.50 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).

Figure (3) shows the effect of IAA on the rooting percentage of gardenia shoots cultured on half strength MS medium. It can be noticed that the high concentration of IAA (12 mgl^{-1}) gave the highest response percent (100%) as compared with the other concentrations. The minimum concentration of auxins failed to promote root initiation to an adequate level, this may be due to failing to elevate auxins concentration to the suitable level for pushing the cells toward elongation and dedifferentiation and convert them to roots primordial (Peak *et al*., 1987). These results are in agreement with those found by Moretti *et al*. (1991).

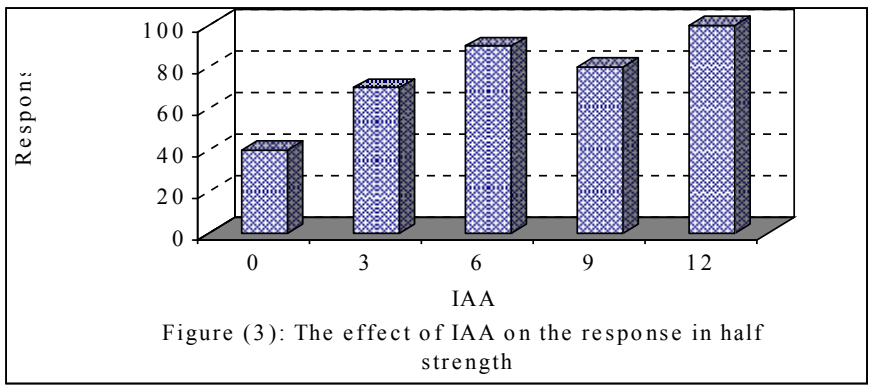


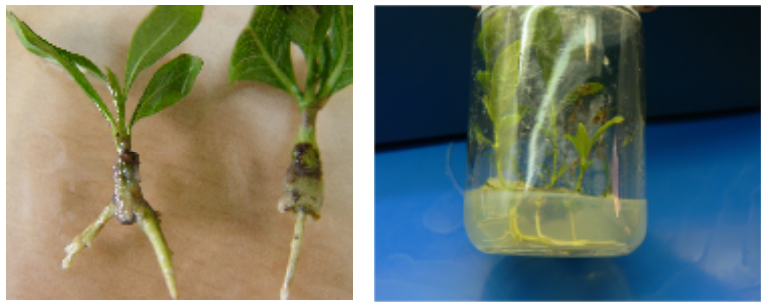
Figure (3): The effect of IAA on the response in half strength

Table (4) shows the effect of IAA concentrations on the average root numbers and lengths /shoot. It is clear that the high concentration of IAA (12 mgl^{-1}) produced more root and increase root lengths /shoot (3.4, 3.3cm respectively). The optimum hormonal balance to push the roots to grow and develop in the presence of exogenous hormones will promote root formation on shoot bases (George and Shermington, 1984). These results are in agreement with those found by Werner and Boe (1980).

Table (4): Effect of IAA on number and roots length on shoots planted in half strength MS media.

Treatment mg l^{-1} (IAA)	Average No. of Roots	Average Length of Roots
0	0.80 b	0.70 c
3	3.00 a	1.90 b
6	2.40 a	2.10 b
9	3.20 a	2.70 ab
12	3.40 a	3.30 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).



(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: 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. 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. 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 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), and chestnut (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.

الاكثار الدقيق لنبات الكاردينيا *Gardenia jasminoides* باستخدام العقد المفردة

مصلح محمد سعيد دهوكي ختام اديب رشيد
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الخلاصة

نفذت التجربة في مختبر زراعة الانسجة النباتية – كلية الزراعة جامعة دهوك للمدة من ٦-٢٠٠٦ الى ١٢-٢٠٠٧ وذلك لاكثار نبات الكاردينيا خارج الجسم الحي باستعمال عقد مفردة حاوية على براعم ابطية ووسط MS نصف القوة المجهز بتراكيز مختلفة من الاوكسينات والسايتوكاينينات اظهرت النتائج في مرحلة النشوء ان اعلى معدل لعدد الفروع كان عند الزراعة على وسط MS بنصف قوة املاحه المجهز ب ٥ ملغم /لتر BA وتم الحصول على اعلى معدل لعدد الفروع والاوراق واطوال النموات عند الزراعة على وسط MS بنصف القوة والمجهز ب ٠.٤ ملغم/لتر IAA .وبالنسبة لتداخلات كان افضل هذه التداخلات في اعطاء النموات الخضرية هي الاوساط المزودة ب٢ملغم /لتر BA + ٠.٤ ملغم /لتر IAA حيث تم الحصول على اكبر معدل لعدد الفروع والاوراق واطوال النموات .اما بالنسبة لمرحلة التضاعف الخضري ان اعلى معدل لعدد الفروع والاوراق واطوال النموات تم الحصول عليها عند الزراعة على وسط MS بنصف القوة المجهز ب ٢ ملغم /لتر BA .وتم الحصول على اكبر معدل لعدد الفروع واطوال النموات على الوسط المجهز ب ٠.٣ ملغم/لتر IAA .وبالنسبة لتداخلات فان المعاملة ٢ملغم /لتر BA +٠.٢ ملغم /لتر IAA اعطت اعلى معد ل لعدد الاوراق واطوال النموات .اما بالنسبة لمرحلة التجذير اظهرت النتائج ان اعلى نسبة مئوية لتجذير ٩٠% كانت عند الزراعة الافرع على وسط MS بصف قوة املاحه المجهز ب ٨ ملغم /لتر NAA وايضا اعطى هذا التركيز اعلى معدل لعدد الجذور وطول الجذور وكذلك بلغت نسبة التجذير ١٠٠% عند زراعة الافرع في وسط MS نصف قوة املاحه والمجهز ب١٢ ملغم /لتر IAA وايضا اعطى هذا التركيز اعلى معدل لعدد الجذور وطول الجذور. كما امكن وبنجاح اقلمة نباتات الناتجة من الزراعة النسيجية ونقلها الى التربة و بلغت نسبة النجاح ٩٥%.

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