

PROPAGATION OF *Gardenia jasminoides* ELLIS BY USING TISSUE CULTURE TECHNIQUES

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ABSTRACT

The experimental trial was consummated in the Plant Tissue Culture Laboratory at El-Zohria Botanical Garden, Horticulture Research Institute, Agriculture Research Center, Ministry of Agriculture from 2010 to 2012. The aim of this study was to reach a well defined protocol for *in vitro* propagation of *Gardenia jasminoides* Ellis plant. Shoot tips and auxiliary buds of *G. jasminoides* were surface sterilized with 30% Clorox (®sodium hypochlorite as commercial bleach) for 25 min which gave the best results (100 % survival, 0.0 % contamination and (0.0 %) mortality of shoot tips and auxiliary buds. Explants were cultured on MS medium supplemented with 4.0 mg/l NAA for effective establishment . In the multiplication stage, 5.0 mg/l BA was favoured for the number of shoots. For *in vitro* rooting, 5.0 mg/l IBA was more suitable to form roots on *G.jasminoides*. After one month in a rooting medium, gardenia plants were transplanted into pots containing peatmoss and covered by polyethylene sheets. After one month, the sheets were removed and left for two weeks under plastic house conditions where 100 % of the plants survived.

Key words: *gardenia, in vitro .micropropagation, shoot tips ,tissue culture.*

1. INTRODUCTION

Gardenia is an indoor ornamental plant. The flower has an attractive appearance accompanied with very nice fragrance. The plant is a source for the pigment carotene. Propagation of *Gardenia* is a difficult task.

Minas (2007) demonstrated that *Gardenia* plants were taken to the laboratory, 10 apical growing shoots, 5 cm long, were removed from each plant and sterilised by soaking in 20% commercial bleach solution with a few drops of Tween 20 for 10 min and washed 3 times in distilled sterile water. This treatment was the most suitable for surface sterilization.

Shen (1990) reported that *G. jasminoides* was cultured on MS medium supplemented with 2 mg/l BA plus 0.1 mg/l IAA, then sub cultured on medium containing 3 mg/l BA plus 0.05 mg/l IAA at the establishment stage. Mir *et al.* (2010) demonstrated that BAP was the most effective cytokinin for the induction of shoots and synergistic effect between BAP and auxins was exhibited .It has a positive effect in the induction of shoots in *G. gummifera*. MS basal medium with

1 mg/l BAP and 0.5 mg/l IBA yielded the highest number of shoots.

Mir *et al.* (2009) reported that explants of *G. gummifera* were cultured on MS medium with appropriate supplement of plant growth regulators. MS medium supplemented with BAP 2 mg/l and NAA 0.5 mg/l elicited the maximum number of shoots from nodal explants.

Pontikis (1983) found that rooting of *G. jasminoides* was achieved by transferring the shoots to a medium containing 1 mg/l IBA and without BA. Dumanois *et al.* (1984) showed that rooting of *Gardenia jasminoides* occurred easily in 3 weeks *in vitro* in the presence of IAA (1 mg/l) and charcoal (2 g/l) or *in vivo* by soaking the base of the shoot in the same auxin for 2 h. George *et al.* (1993) found that efficient rooting of *G. jasminoides* Ellis was achieved on MS medium supplemented with NAA at 5 mg/l. Abdullah *et al.* (2003) demonstrated that the highest rooting percentage (up to 98%) was obtained as a result of reducing the mineral salt concentration to half, 20 g/l sucrose, 2 g/l activated charcoal (AC) and 1 mg/l IAA.

Economou and Spanoudaki (1986) found that microcuttings of gardenia harvested from cultures grown in a medium with 2iP or 2iP plus GA₃ rooted readily in a peat:perlite mixture at 2:1 v/v.

The aim of this study was to investigate the best protocol for *in vitro* propagation of *G. jasminoides* Ellis for commercial production. Moreover, to determine and devise simple and reliable methods for *in vitro* propagation through inducing growth. The current experiments were achieved on *Gardenia jasminoides* Ellis to investigate the effect of various media ingredients on shoot proliferation and rooting response of explants *in vitro*.

2. MATERIALS AND METHODS

This study was carried out in the Laboratory of Tissue Culture, Zohria Botanical Garden, Cairo, Egypt, Horticulture Research Institute, Agriculture Research Center, Ministry of Agriculture, Egypt.

The experiments were carried out during the period from (2010-2012), to study the most suitable protocol for micropropagation of *G. jasminoides* Ellis.

2.1. Plant material

The mother plants were grown under greenhouse conditions at Zohria Botanical Garden. Shoot tips and axillary buds were used as explants.

2.2. Culture Room Conditions

Cultures of *G. jasminoides* Ellis were placed in growth chambers under 25±2 °C and 16-h photoperiod provided with white fluorescent light and 2000 lux.

2.3. Culture Media

Murashige and Skoog (MS) medium was used for explants of *G. jasminoides* Ellis. Media were solidified and supplemented with 7.0 g/l agar. Sucrose at 30.0 g/l was added as a source of carbohydrate. The pH of the medium was adjusted to 5.7. Twenty ml medium were poured in 150 ml jars and sterilized by autoclaving under steam pressure 1.5 bar at 121°C for 20 min. Each treatment consisted of 3 jars. In each jar, three shoots were cultured separately.

2.4. Experimental Treatments

2.4.1. Surface Sterilization of Explants

Shoot tips of gardenia plants were excised from the mother plants, initially 1-2 cm in length, washed by soapy water for 5 minutes, followed by 1 hour under running tap water. The explants were surface sterilized by immersion in a Clorox (sodium hypochlorite) solution as a commercial bleach at 20, 30 and 40 % containing 3-5 drops of Tween 20 for 15, 20, 25 and 30 minutes. Finally,

they were washed 5 times with sterile distilled water. Each sterilized explant was cultured separately under sterile conditions in 100 ml jar and each treatment consisted of three jars with three individual shoot in each jar.

2.4.2. Establishment Stage

Four treatments were initiated using NAA at 0.0, 1.0, 2.0 and 4.0 mg/l for the establishment stage. In each treatment nine explants in three replicates were cultured for one month. Finally, the shoot length and the number of leaves were recorded.

2.5. Multiplication Stage

For the multiplication stage, seven treatments were initiated with BA at different concentrations (0.0, 0.5, 1.0, 2.0, 3.0, 4.0 and 5.0 mg/l BA). This stage was repeated three times by subculturing on the same media treatments. At the end of the experiments, the collected data included the number of shoots, shoot length and the number of leaves.

2.6. Rooting Stage

For the rooting stage, four treatments were used with IBA at different concentrations (0.0, 1.0, 3.0 and 5.0 mg/l IBA). After one month, the number of leaves, shootlet length (cm), the number of roots and root length (cm) were scored.

2.7. Acclimatization Stage

Rooted plantlets were pricked out singly into 5 cm (in diameter) plastic pots filled with 1:0, 1:1 and 1:2 (v/v) peatmoss and sand, respectively. The potted plantlets were kept in the green house. To maintain cultures at high humidity, pots were covered with clear transparent plastic sheets for one month. The plastic covers were then gradually removed to reduce humidity and to adapt plantlets to greenhouse conditions. At the end of the experiments, the collected data included shootlet length and number of leaves were recorded.

2.8. Experimental Design and Statistical Analysis

A factorial experiment in a complete randomized design was employed in all the experiments. Analysis of variance was used to show statistical differences between treatments using L.S.D at 5% probability level (Snedecor and Cochran, 1989).

3. RESULTS AND DISCUSSION

3.1. Effect of Clorox concentrations and soaking periods on the survival percentage of surface sterilization for *Gardenia* explants

For surface sterilization of *G. jasminoides*, data in Table (1) revealed that the effect of Clorox

was positive in survival percentage. The highest survival percentage was recorded at 30% Clorox @concentration.

For soaking period, the data indicated that increasing the time of immersing the explants the survival percentage increased. The highest percentage of survival explants was recorded with immersion for 25 min.

The interaction between Clorox and time was significant with the highest percentage of survived explants (100), with 30% clorox for 25 min.

The results obtained are in harmony with those obtained elsewhere when 70% alcohol (ethanol) and 30 % Clorox were used alone (Hosni, 2001).

Table (1): Effect of different concentrations of Clorox and socking period on explants survival percentage of gardenia.

Min Clorox \	15	20	25	30	Mean (A)
20	44.44	55.55	55.55	66.67	55.55
30	55.55	66.67	100	77.78	75.00
40	66.76	66.67	55.55	44.44	58.34
Mean(B)	55.55	62.97	70.37	62.96	

L.S.D at 5% A=11.44 B=14.30 A*B =22.87

3.2. Effect of Clorox concentrations and soaking periods on mortality percentage of surface sterilization for Gardenia explants

The results demonstrated in Table (2) indicate that surface sterilization by Clorox was positively significant on mortality of *Gardenia* explant. This effect increased with the increase of clorox concentration. The best concentration was 20 %, which gave 0.0 mortality explants.

Concerning soaking period, data showed continuous additive increases in mortality percentage due to the increase in immersed soaking period. The highest percentage of mortality explants was recorded when the explants were soaked for 15 min, which gave 3.70 percentage of mortality explants.

Table (2): Effect of Clorox concentrations and soaking periods on mortality percentage of surface sterilization for gardenia explants.

Min Clorox \	15	20	25	30	Mean (A)
20	0.00	0.00	0.00	0.0	0.00
30	0.00	11.11	0.00	22.22	8.33
40	11.11	33.33	33.33	55.55	33.33
Mean (B)	3.70	14.81	11.11	25.94	

L.S.D 5% A=9.82 B=4.35 A*B= 18.72

For the interaction between clorox concentrations and soaking periods, the use of clorox was lower at 20 % clorox for different times.

3.3. Effect of Clorox concentrations and soaking periods on contamination percentage of surface sterilization for Gardenia explants

Data in Table (3) also show that clorox caused a decrease in contamination percentage of *Gardenia*. This was true between the different concentrations of clorox used and also when compared with the lowest concentration.

On the other hand, the data indicated that increasing the time of immersing the explants decreased the contamination percentage of. While, the best time achieved for free contaminated explants was 30 min.

For the interaction, the results showed that the highest percentage of free contaminated explants were achieved at 30% clorox for 25, while the lowest value of free contamination explants was found at 20 % clorox for 15 min.

For the interaction between clorox concentrations and soaking period, data in Tables (1, 2 and 3) indicated that the best percentage of explant survival and contamination free explants were (100.0 and 100.0%) were observed when explants immersed for 25 min in 30% clorox. Obtained results showed that the lowest mortality percentage was that contained 30% Clorox for 25 min (0.00 %).

Table (3): Effect of Clorox concentrations and soaking periods on contamination percentage of surface sterilization for gardenia explants.

Min Clorox \	15	20	25	30	Mean (A)
20	55.55	44.44	44.44	33.33	44.44
30	44.44	22.22	0.00	0.00	16.66
40	22.22	0.00	11.11	0.00	8.33
Mean(B)	40.74	22.22	18.22	11.11	

L.S.D at 5% A=11.43 B=13.20 A*B=22.87

3.4. Effect of different concentrations of NAA on the establishment stage of Gardenia

The results in Table (4) indicated that the best NAA concentrations were 2.0 and 4.0 mg/l. The results showed quite clearly that the highest shoot length was 3.33 and 3.66 cm at 2.0 and 4.0 mg/l NAA, respectively. Also, the same concentrations of NAA showed the highest value of the number of leaves (7.66 and 8.33). However, the explants cultured on hormone free MS-medium (control) gave the shortest shootlet length

(0.44 cm) and the lowest number of leaves (4.33 leaves).

These results are in line with others obtained with different species like *Gerbera jamesonii* (Aswath & Choudhary, 2002 and Kumar *et al.*, 2004). On the other hand, MS medium proved to be superior to B5 medium (Chikhale *et al.*, 2004).

Table (4): Effect of different concentrations of NAA(mg/l)on the establishment stage of *Gardenia jasminoides* .

NAA(mg/l)	Shoot Length cm	No. of leaves
0.0	0.44	4.33
1.0	1.83	5.33
2.0	3.33	7.66
4.0	3.66	8.33
L.S.D at 5%	0.56	0.40

3.5. Effect of different concentrations of BA and subculturing on the number of shoots for multiplication stage of *Gardenia*

For the number of shoots, the data calculated in Table (5) show that BA concentrations induced an increase in the number of shoots when compared with the control in each case. However, there were no significant differences in the number of shoots in the two higher levels of BA (4.0 and 5.0 mg/l).

Concerning the effect of repeating the subculture, and regardless of the effect of various BA, it was evident that repeating subculture increased significantly number of shoots.

Regarding the interaction between BA and number of subculture, after three subcultures, the shoots were cultured on MS-medium supplemented with 4 mg/l BA produced the highest number of shoots. While the shoots in the first subculture on free hormone MS medium (control) did not produce any shoots.

On the other hand, BA at 0.5 mg/l was superior to 1.0, 2.0 or 4.0 mg/l when compared to the explants from the leaves of *Gerbera* (El-Shamy *et al.*, 2009).

3.6. Effect of different concentrations of BA and subculturing on shoot length for multiplication stage of *Gardenia*

For the shoot length, data in Table (6) showed that raising the concentration of BA resulted in a significant decrease in shoot length (8.67cm control treatment). While the lowest value of shoot length (4.67 cm) was recorded with 5.0 mg/l BA.

For the effect of repeating the subculture on shoot elongation, it was found that elongation occurred and increased positively as time elapsed.

The interaction between the effect of BA and repeating the subculture, the elongation showed that 0.0 mg/l BA at the third month, the longest shoots (8.67 cm) were obtained. Also, there were significant differences in shoot length between the different concentrations of BA and repeating the subculture.

3.7. Effect of different concentrations of BA and subculturing on the number of leaves for multiplication stage of *Gardenia*

Results for the number of leaves presented in Table (7) showed that BA decreased the number of leaves probably due to a decrease in stem elongation and in the number of internodes. Thus, it was found that the highest the number of leaves was obtained and maintained at zero-level BA (16.57) when compared to the higher BA concentrations (0.5, 1, 2, 3, 4 and 5 mg/l).

Concerning the number of leaves, data exhibited in Table (5) indicate that there were continuous additive decreases in the number of leaves due to the increase repeating the subculture. They were decreased as a result of repeating the subculture.

Table (5): Effect of different concentrations of BA and subculturing on the number of shoots for the multiplication stage of *Gardenia*.

BA mg/l	Subculture 1	Subculture 2	Subculture 3	Mean(A)
0.0	1.00	3.33	6.66	3.56
0.5	2.66	5.66	9.33	5.88
1.0	3.66	7.66	10.66	7.33
2.0	4.66	8.66	11.33	8.22
3.0	5.33	9.33	11.66	8.77
4.0	7.66	10.33	13.33	10.44
5.0	8.00	10.66	13.66	10.77
Mean(B)	4.71	7.95	10.95	

L.S.D at 5% A=0.52 B=0.34 A*B=0.90

Table (6): Effect of different concentrations of BA and subculturing on shoot length for the multiplication stage of gardenia.

BAmg/l	Subculture 1	Subculture 2	Subculture 3	Mean(A)
0.0	4.16	5.83	8.66	6.28
0.5	3.83	5.16	6.50	5.17
1.0	3.83	5.00	6.16	5.00
2.0	3.66	4.83	5.83	4.77
3.0	3.50	4.66	5.66	4.61
4.0	3.33	4.33	4.83	4.18
5.0	3.16	3.83	4.66	3.88
Mean(B)	3.64	4.81	6.07	

L.S. D at 5% .A=0.31 B= 0.20 A*B=0.5390

Table (7): Effect of different concentrations of BA and subculturing on the number of leaves for the multiplication stage of gardenia.

BAmg/l	Subculture 1	Subculture 2	Subculture 3	Mean(A)
0.0	11.66	17.00	21.00	16.57
0.5	10.33	15.00	17.66	14.33
1.0	9.66	12.00	16.00	12.78
2.0	8.66	11.66	14.33	11.56
3.0	7.00	10.00	12.33	9.78
4.0	6.66	9.00	11.00	8.89
5.0	6.00	9.00	9.66	8.22
Mean(B)	8.57	12.048	14.57	

L.S.D at 5% A=0.79 B=0.52 A*B=1.38

For the interaction between the effect of BA and repeating the subculture on the number of leaves, the results showed that the number of leaves started to decrease from the first subculture and was more or less stable until the third subculture. Higher number of leaves was recorded at the third subculture (21) and the control treatment.

3.8. Effect of different concentrations of IBA and activated charcoal on shoot length for rooting stage of Gardenia

The results presented in Table (8) show that at the different concentrations of IBA, the longest shootlet (11.33 cm) was produced when MS medium contained 3.0 mg/l IBA. But the shortest shootlet length (7.92 cm) was recorded when the shoots were cultured on free hormone MS medium (control).

For activated charcoal, the highest shootlet length (10.75 cm) was formed when MS medium contained activated charcoal, while the shortest value of shootlet length (9.25 cm) was formed at MS medium without activated charcoal.

Also the results exhibited in Table (8) showed that MS medium containing 3.0 mg/l IBA with activated charcoal caused an increase in shootlet length (12.17 cm). This was true between the different concentrations of IBA with activated

charcoal and also when compared with the zero-level control.

3.9. Effect of different concentrations of IBA and activated charcoal on the number of leaves for rooting stage of Gardenia

Regarding IBA addition, data in Table (9) revealed that after rooting stage, the mean number of leaves was 25.17 at 3.0 mg/l IBA which was highly significant when compared with 0.0 mg/l IBA.

Activated charcoal was added in a separate rooting stage experiment with IBA at the same concentration used previously in order to improve the state of shootlets. As expected, improvement was observed in developing shootlets with respect to colour and the number of leaves.

Data of the interaction between IBA and activated charcoal showed quite clearly that the highest number of leaves (27.33) was formed at 3.0 mg/l IBA plus activated charcoal.

3.10. Effect of different concentrations of IBA and activated charcoal on the number of roots for rooting stage of gardenia

Data in Table (10) revealed that supplementation of MS medium with activated charcoal and IBA at 0.0, 1.0, 3.0 and 5.0 mg/l increased significantly the number of roots. IBA at 5.0 mg/l showed the highest

Table (8): Effect of different concentrations of IBA and activated charcoal on shoot length for rooting stage of Gardenia.

IBAmI/l	With AC	Without AC	Mean(A)
0.0	8.66	7.16	7.92
1.0	10.33	9.16	9.75
3.0	12.16	10.50	11.33
5.0	11.83	10.16	11.00
Mean(B)	10.75	9.25	

L.S.D at 5% A=0.500 B=0.354 A*B= 0.707

Table (9): Effect of different concentrations of IBA and activated charcoal on the number of leaves for rooting stage of Gardenia.

IBAmI/l	With AC	Without AC	Mean(A)
0.0	22.33	18.66	20.50
1.0	23.66	19.33	21.5
3.0	27.33	23.00	25.17
5.0	25.00	22.23	23.67
Mean(B)	24.58	20.83	

L.S.D at 5% A=1.060 B=0.749 A*B=1.499

Table (10): Effect of different concentrations of IBA and activated charcoal on root length for rooting stage of Gardenia.

IBAmI/l	With AC	Without AC	Mean(A)
0.0	3.33	0.00	1.67
1.0	4.50	0.00	2.25
3.0	6.66	3.16	4.92
5.0	10.00	3.83	6.92
Mean(B)	6.12	1.75	

L.S.D at 5% A=0.395 B=0.279 A*B=0.558

number of roots (12.83).

The addition of activated charcoal caused an increase in the number of roots of Gardenia plantlets as compared with the zero-level (control). The highest number of roots was found when activated charcoal was used.

The interaction between the different concentrations of IBA and activated charcoal was in general, significant in increasing the number of gardenia roots. The best medium was MS medium containing 5.0 mg/l IBA (16.33), with activated charcoal which led to the greatest number of shoots.

These results are in line with Amo-Marco and Ibanez (1997) on *Limonium advanillesii* and Matsumato *et al.* (1998) on hybrid *Statice* var. "Blue Symphonet".

3.11. Effect of different concentrations of IBA and activated charcoal on root length for rooting stage of Gardenia

Data in Table (11) revealed that the shoots Cultured on MS medium supplemented with 5

mg/l IBA produced the longest roots (6.92 cm). But, the shoots cultured on free hormone MS medium gave the shortest roots (1.67 cm).

For activated charcoal, the highest root length was observed in MS medium supplemented with activated charcoal (6.13 cm) compared to the medium without activated charcoal which gave 1.75 cm.

Regarding the interaction between IBA concentrations and activated charcoal, shoots cultured on MS medium containing 5 mg/l BA and activated charcoal produced the longest roots (10 cm). While, the shoots on free hormone MS medium (control) gave no roots.

3.12. Effect of different concentrations of peatmoss and sand on acclimatization stage of Gardenia jasminoides.

All rooted shoots were acclimatized on peatmoss and sand. The survival of plantlet after one month was calculated and gained 100% percentage (Table 12).

Table (11): Effect of different concentrations of IBA and activated charcoal on the number of roots for rooting stage of Gardenia.

IBAm/l	With AC	Without AC	Mean(A)
0.0	2.66	0.00	1.33
1.0	4.66	0.00	2.33
3.0	9.33	0.33	7.83
5.0	16.33	9.33	12.83
Mean(B)	8.25	3.92	

L.S.D at 5% A =0.865 B=0.612 A*B=1.224

Table (12): Effect of different concentrations of peatmoss and sand on acclimatization stage of *Gardenia jasminoides*.

Peatmoss: sand(v/v)	Shootlet length	No.of leaves
1:0	14.66	14.66
1:1	12.66	11.66
1:2	15.33	17.00
L.S.D at 5%	n.s	n.s

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إكثار نبات الجاردينيا (*Gardenia jasminoides*) بواسطة تقنيات زراعة الأنسجة

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ملخص

أجريت هذه الدراسة في خلال الفترة من سنة 2010- 2012 في معمل زراعة الأنسجة التابع لمعهد بحوث البساتين - مركز البحوث الزراعية-وزارة الزراعة- جمهورية مصر العربية. يهدف هذا البحث إلى معرفة أنسب المعاملات لإكثار نبات الجاردينيا عن طريق زراعة الأنسجة وذلك لوضع بروتوكول للإكثار الدقيق لهذا النبات. ويمكن تلخيص أهم النتائج التي أمكن التوصل إليها في الآتي:
أمكن إكثار نبات الجاردينيا بواسطة القمة النامية والبراعم الجانبية كمنفصلات نباتية حيث تم تعقيمها بواسطة الكلوركس بتركيز 20، 30، 40 % لمدة 15 ، 20 ، 25 ، 30 دقيقة بالإضافة إلى التداخلات بينها . تبين أن أفضل تركيز من الكلوركس للحصول على أعلى نسبة بقاء للنباتات وأقل نسبة تلوث هي 30 % لمدة 25 دقيقة. وبالنسبة لتعقيم الأوراق كان 30 % لمدة 15 دقيقة.
أستخدمت في مرحلة التأسيس بيئة موراشيجي وسكوج المضاف إليها نفتالين حمض الخليك بتركيز صفر، 1.0، 2.0، 4.0 مجم/لتر. فكان أفضل تركيز هو 4.0 مجم/لتر نفتالين حمض الخليك لتكوين فرع جيد.
أستخدمت في مرحلة التضاعف. بيئة موراشيجي وسكوج المضاف إليها البنزويل ادينين بتركيز صفر و 0.5 و 1.0 و 2.0 و 3.0 و 4.0 و 5.0 مجم/لتر. فكان أفضل تركيز هو 5.0 مجم/لتر.
أستخدمت في مرحلة التجذير بيئة موراشيجي وسكوج المضاف إليهما إنول حمض البيوتريك بتركيز صفر، 1.0، 3.0، 5.0 مجم/لتر في وجود الفحم النباتي أو عدم وجوده. فتبين أن أفضل معاملة هي بيئة موراشيجي وسكوج المضاف إليها 5.0 مجم/لتر إنول حمض البيوتريك في وجود الفحم النباتي.
أستخدم في مرحلة الأقامة بيتيموس ورمل بنسبة 1:1 و 2:1 و 3:1 تحت أنفاق بلاستيكية داخل صوب بلاستيكية فكانت نسبة نجاحها 100% في كل المعاملات.

المجلة العلمية لكلية الزراعة - جامعة القاهرة - المجلد (64) العدد الثالث (يوليو 2013): 303- 310.