

The Effect of Growth Hormone in Increasing Flowering of *Gardenia Jasminoides L.*

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Abstract

Gardenia plants are characterized by the beauty of leaves and aromatic flowers, that makes them one of the most important types of ornamental plants. Traditional propagation methods such as seeds and cuttings are considered insufficient to achieve high propagation rates, for this reason, plant tissue culture techniques were used. This study was conducted in Plant Tissue Culture Laboratory of College of Agriculture at University of Kufa, during period from September 5, 2024 to March 5, 2025. Plant parts were used, represented by (shoot tips) and (nodal segments), from the shady (dwarf) variety *Cardinia* known as (*Radicans*). The effect of Benzyl Adenine (BA) concentrations on emergence of seedlings appeared from planting shoot tips of *Cardinia* plant after (4) weeks of cultivation. The 1 mg/L treatment was most effective, that recording highest shoot length (11.0 cm) and number of shoots (1.9), while 0 mg/L treatment achieved highest number of leaves (3.1), but had lower branch length (0.40 cm). The 0.5 mg/L treatment also showed good response, achieving high leaf width (2.1 cm) despite decrease in number of shoots to 1.2. In contrast, the 1.5 mg/L treatment showed an increase in number of branches (2.0) and number of leaves (3.9), but recorded lower shoot length (0.60 cm). On the other hand, 2 mg/L treatment was the least effective, recording lowest number of leaves (0.04) and shoot length (0.10 cm). While the effect of different concentrations of (BA) on multiplying *Gardenia* plants was shown after 12 weeks of cultivation, and concentration 1.5 mg/L recorded highest number of shoots (4.5) and highest number of leaves (4.55), which indicating the effectiveness of this concentration in promoting growth, while concentration of 2.5 mg/L was lowest, recording lowest number of shoots (0.55) and lowest number of leaves (1.85). As for effect of Naphthalene Acetic Acid (NAA) concentrations on emergence of plantlets from tips of *Gardenia* plant after (4) weeks of cultivation, highest response was shown at concentration of 2.5 mg/L, where a 100% response rate was recorded with shoot lengths of (10.3) cm and number of leaves of (39). While the concentration of 0 mg/L was lowest, as no response or growth was recorded. While effect of (NAA) concentrations on growth of *Gardenia* plant was shown after (12) weeks of planting, plants at concentration of 0 mg/L did not show any response, as all values were zero. With increasing concentration to 0.5 mg/L, a complete response (100%) was shown, with production of 5.12 shoots and branch length of (1.65) cm. At concentration of 1 mg/L, the number of shoots decreased to (4.50), Although the response remained complete.

Keywords: *Gardenia*, Growth Hormone, Flowering, Propagation, Rooting, Auxin, Abscisic acid.

I. Introduction

Gardenia plant belongs to the family Rubiaceae .The genus *Gardenia* includes more than 200 species, the most important of which are two species *Gardenia jasminoides* and *Gardenia thunbergia*. The first type, the most important of two, is native to China and is also called *florida.G*. It has many varieties, as for second type, it is native to South Africa. *Gardenia* is considered medium-growing evergreen shrub, with height ranging between 60 and 240 cm, depending on types. It is distinguished by dark green leaves that are characterized by an alternating arrangement and an elongated oval shape (1).



Gardenia produces waxy, fragrant white flowers, single or double, whereas age ranges from three days, that color turns from white to yellow with age. The fruit is an oval-shaped, orange capsule containing the seeds. Gardenia has many uses, the most important for ornamental purposes. It is grown in gardens as beautiful shrub with fragrant flowers. Also used as flowering potted plant, and valuable essential oil is extracted from flowers. Fruits also used to give natural color to foods (2). This plant can be grown in various types of gardens due to the beauty of leaves and fragrant white flowers. Also used as flowering plant, balcony plant, or in interior design. And used flowers to make bouquets that decorate vases, making it one of the most important cut flowers in America and many European countries (3).

Gardenia tree was introduced to Iraq for first time in 1959, where it was cultivated at Al-Zafaraniya farm (4). Gardenia prefers moist environments with well-drained soil and pH that tends to be acidic (5). If the soil pH exceeds 6.5, this may lead to deficiency in micronutrients, especially iron, causing yellowing of the leaves (chlorotic). Gardenia is one of the most prominent ornamental plants spread worldwide, distinguished by the beauty of its flowers, which are considered among the most important cut flowers in America and many European countries (6). Gardenia belongs to the genus *Gardenia* and the Rubiaceae family, with *G. jasminoides* being the most widespread and important species (7). Gardenia is grown in tropical and subtropical regions (8). From annual observations of gardenia grown in gardens under Iraqi conditions, flowering is observed in some seasons at expense of others. It faces problem of not forming flower buds, flower buds falling off, and short flower life. It is plant that is difficult to grow, even for professional breeders. Gardenia behaves like facultative short-day plants to form flower buds and needs day length of more than 12 hours for flower buds to develop, taking into account temperature of no less than 11°C.

Gardenia is an evergreen, branching shrub that plays an important role in landscaping. It is characterized by dark green, glossy leaves with prominent veins, in addition to white flowers, which are significant source of fragrant scent (9). This shrub can be used as ground cover, or as an indoor or outdoor ornamental plant, where it grows in full sun, which is considered essential for achieving the best flower production. (10).

Gardenia is propagated by traditional methods such as seeds and young cuttings. However, it is preferable not to propagate by seed methods due to (Heterozygous) and phenotypic variation of resulting individuals. that is undesirable for agricultural purposes. The primary aim of seed propagation is to produce new cultivars through hybridization or production of rootstocks for grafting, and this method is not used for productive purposes (11).

Traditional methods of propagating gardenia result in low propagation rates, while plant tissue culture techniques can achieve high propagation rates through organ formation. Where Auxin (MS) added with concentration 0.8%, which achieved highest rooting rate (75%) in the laboratory for type when using concentration 0.6 (NAA). (12).

The young leaves, petals and growing tips of Gardenia plant were grown on medium MS, supplemented with different concentrations of Auxin and Cytokinin, and observed the best vegetative growth (3.2–1.6–0.8 BA) when using high concentrations of cytokinin, while the best root formation was achieved when concentration was reduced to 0.4 μM (13), and obtained good quality plants, not transparent, and to face the conditions of regionalization, when cultured in unsealed tubes, using an appropriate concentration of sucrose (3%), and incubating under appropriate light conditions and light intensity (14).

II. Materials and Methods

This study was conducted in the Plant Tissue Culture Laboratory of College of Agriculture at University of Kufa, during the period from September 5, 2024 to March 5, 2025. Plant parts, including (Shoot tips) and (Nodal segments), from the shady (dwarf) variety *Cardinia* known as 'Radicans'

These parts were excised using sharp scalpel, with dimensions of approximately 1.5 cm. These parts were grown in anvils imported from the Netherlands



The plant parts were washed with soap and water several times to get rid of dust and suspended materials, then washed with distilled water several times and immersed in glass container containing 500 mg/L of fungicide (Elsa) with added 3 drops of liquid soap. This mixture was stirred several times for 5 minutes, and then the parts were washed with sterile distilled water several times (15)

All plant tissues of each specie were preserved separately in glass jars containing an antioxidant solution, which consisted of 150 mg/L citric acid and 100 mg/L ascorbic acid, and keep the tissues in refrigerator at 4°C for 24 hours even performing the surface sterilization process.

After removing from antioxidant solution, the parts were immediately placed in 70% ethyl alcohol solution for 5 minutes and then washed several times with distilled and sterile water. The lower parts of both types of plant parts were cut to lengths of 0.5 cm, in order to get rid of sterile material that penetrated to the tissues after sterilization with alcohol, then the parts were immersed in 40% sodium hypochlorite solution (prepared from commercial solution containing 1.05% active ingredient of sodium hypochlorite with addition 3 drops of spreading agent (Tween-20) for 30 minutes with stirring occasionally. After that, plant parts were extracted and washed in distilled and sterile water several times, then transferred to another solution containing sterilizing agent (mercuric chloride) in concentration 0.1% for 10 minutes, then it was washed with distilled and sterilized water several times to get rid harmful sterilizing substance (16).

2-1 Preparing the nutrient medium:

Use nutrient medium (MS) consisting of salts and organic materials, where weight of 4.33 g/L was taken from the following components:

- Sucrose at concentration 30 g/L
- Adenine sulfate at concentration 100 mg/L
- Myosoinositol at concentration 80 mg/L
- Sodium phosphate at concentration 170 mg/L
- Some vitamins and glycine at concentration 1 mg/L

Then pH of nutrient medium was adjusted to 5.8, and added activated charcoal at concentration 5 g/L, and agar at concentration 250 mg/L. The medium was heated to 90°C and then dispensed into 2.5 cm × 18 cm tubes, 20 ml each. The tube mouths were sealed with cotton and covered with aluminum foil. Each treatment contained ten test tubes. Also nutrient medium was distributed into glass bottles measuring 14 cm x 6.5 cm, with five replicates for each treatment, and cultured in each tube one plant part. After that tissues were sterilized using an autoclave under pressure 1.04 kg/cm² and temperature 121°C for 20 minutes. After sterilization cultures were incubated under suitable growth conditions.

In the growth chamber the temperature was set at 25°C with light 1000 cd/ft for 16 hour per day. This study included several experiments, including:

1-1-2 First Experiment: The effect of plant part source on the emergence of plantlets:

Cytokinin AB was used at different levels (0, 0.5, 1.0, 1.5, 2.0, 2.5 mg/L) with GA3 and NAA at constant concentration of 0.2 mg/L, then study effect of these treatments on the vegetative shoots of two types of dwarf gardenia (*Gardenia jasminoides* Radicans) Where used:

- Shoot tips
- Nodal segments

After sterilization of plant parts as mentioned previously, they were placed in nutrient medium (MS) supplemented with salts, where one plant part was cultured in each test tube. The cultures were incubated under light conditions for 4 weeks.

2-1-2 Measurements taken during the experiment:

1. Percentage growth response (%).
2. Number of vegetative shoots per plant part.
3. Height of vegetative shoot (cm).
4. Number of leaves per vegetative shoot.
5. Leaf width (cm).

Data was collected and analyzed to evaluate the effect of different treatments on the growth of plant parts. In the propagation stage (second cultivation), then take laboratory growths which resulting from initial cultivation, and divided into precise laboratory cuttings, and grown in new media of nutrient solutions on which initial cultivation succeeded. While the primary cuttings were replanted after taking new growths from them to root or propagate again after leaving the bases of new growths on them which contain the spores for several cultivations at rate of once every 12 weeks due to obtaining stems for propagation (tissue mothers) inside tubes without need to repeat sterilization process and to ensure production of largest number of new leafy growths and to reach commercial production with high economic feasibility.

III. Results:

3-1 Measure the effect of BA concentrations

Laboratory breeding using tissue culture techniques for vegetative propagation has previously been shown to be ideal method for obtaining homogeneous vegetative strains, far from important and essential treatment in the success of this technique, where the effectiveness of these materials varies according to their type, concentration, interaction with each other, and the type of plant material used, and cytokinin (BA) is the most widely used in propagation of kinetin.

The data in Table (1) show that the 1 mg/L treatment was the most effective, recording the highest branch length (11.0 cm) and number of shoots at 1.9. Meanwhile, the 0 mg/L treatment achieved the highest number of leaves (3.1) but the lowest shoot length (0.40 cm). The 0.5 mg/L treatment also showed a good response, achieving a high leaf width (2.1 cm) despite decrease in number of shoots to 1.2. In contrast, the 1.5 mg/L treatment showed an increase in the number of shoots (2.0) and number of leaves (3.9), but recorded lower shoot length (0.60 cm). On the other hand, 2 mg/L treatment was the least effective, recording the lowest number of leaves (0.04) and shoot length (0.10 cm). Finally, the 2.5 mg/L treatment showed significant reduction in growth, with the lowest measurements in all parameters. These results indicate clear effect of treatment concentrations on growth.

Table (1) shows effect of BA concentrations in the emergence of plants from cultivated tips of shoots for Gardenia plant after (4) weeks of cultivation

Treatment BA mg/L	Percentage of Response	Shoots No. Plant Part	Shoot length /cm	leaves No. Plant Part	Leaf Width/cm
0	%100	1.5	0.40	3.1	1.8
0.5	%100	1.2	0.87	1.9	2.1
1	%100	1.9	1.0	2.2	9.0
1.5	%100	2.0	0.60	3.9	0.8
2	%100	1.1	0.10	0.04	0.1
2.5	%100	0.3	0.50	1.3	0.3

*L.S.D. (0.05) = 2.1

3-1-1 Measuring the Effect of BA Concentrations on Cardinia Plant growth after 12 Weeks

Table (2) shows the effect of BA concentrations on Cardinia plant growth after 12 weeks of cultivation, where measured several characteristics, such as number and length of shoots, number and width of leaves. The 1.5 mg/L concentration recorded highest number of shoots (4.5) and the highest number of leaves (4.55), that indicates the effectiveness of concentration in promoting growth, while 2.5 mg/L concentration recorded lowest, where recording lowest number of shoots (0.55) and lowest number of leaves (1.85).

Also data shows that concentration of 0.5 mg/L achieved good results in leaf number (5.10) and shoot length (1.65), suggesting that lower concentrations may also be effective. However, an inverse relationship between high concentrations of BA and their negative effect on plant growth is evident, with performance declining at maximum concentrations such as 2 mg/L and 2.5 mg/L. These results support the idea that an optimal concentration of BA can improve gardenia growth and contribute to increased productivity.

Table (2) shows effect of different concentrations of BA on multiplication of gardenia plants after 12 weeks of Cultivation

Treatment BA mg/L	Percentage of Response	Shoots No. Plant Part	shoot length /cm	leaves No. Plant Part	Leaf Width/cm
0	%100	1.00	2.05	1.30	1.10
0.5	%100	2.25	1.65	5.10	0.65
1	%100	1.75	0.70	3.06	0.95
1.5	%100	4.5	1.05	4.55	1.5
2	%100	3.00	1.55	2.45	0.53
2.5	%100	0.55	0.45	1.85	0.10

L.S.D.= ≈ 1.484

Table (3) shows effect of NAA concentrations on the growth of the shoot tips for gardenia plant after 4 weeks. The highest response was at concentration 2.5 mg/L, where 100% response rate was recorded with shoot lengths 10.3 cm and number of leaves 39, while the concentration 0 mg/L was the lowest, where no response or growth was recorded.

The response ranged from 100% at concentrations from 0.5 to 2.5 mg/L, indicating the effectiveness of NAA in promoting growth. A concentration 1.5 mg/L also achieved good results with shoot lengths 8.5 cm, highlighting the importance of intermediate concentrations. The table shows that higher concentrations promote growth, confirming the positive relationship between NAA concentration and plant growth. The results confirm that higher concentrations



of NAA significantly promote growth, as the calculated LSD value (≈ 1.142) helps to clarify presence of significant differences in results.

3-2 Measuring the effect of NAA Concentrations

3-2-1 Measuring the Effect of NAA Concentrations after (4) Weeks

Table (3) shows effect of NAA concentrations on the emergence of plantlets from cultivated tips of shoots for gardenia plant after 4 weeks of cultivation

Treatment BA mg/L	Percentage of Response	Shoots No. Plant Part	shoot length /cm	leaves No. Plant Part	Leaf Width/cm
0	%100	0.00	0.00	0.00	0.00
0.5	%100	6.25	1.35	4.9	2.10
1	%100	2.9	1.0	2.7	1.9
1.5	%100	8.5	2.3	10.5	0.80
2	%100	4.10	1.10	2.10	1.1
2.5	%100	10.3	1.50	9.3	1.30

L.S.D (0.05) = ≈ 1.142

3-2-2 Measuring the effect of NAA Concentrations after (12) Weeks

The results of Table (4) show the effect of NAA concentrations on the growth of gardenia plants after 12 weeks of cultivation. The table includes six different NAA concentrations, ranging from 0 mg/L to 2.5 mg/L, with data on the percentage response, number of shoots, shoot length, number of leaves, and leaf width. Plants at concentration 0 mg/L showed no response, with all values being zero. Increasing the concentration to 0.5 mg/L, complete response (100%) was achieved, with 5.12 shoots produced and shoot length 1.65 cm. At concentration 1 mg/L, the number of shoots decreased to 4.50, but the response was still complete. A concentration 1.5 mg/L showed the best results, with 10.10 shoots, branch length 0.90 cm, and leaf number 6.50. At 2 mg/L, the number of shoots was 7.30 and the shoot length was 21.00 cm, indicating the effectiveness of concentration in promoting growth. The concentration 2.5 mg/L achieved highest number of shoots (11.50) and the shoot length was 7.00 cm, indicating the success of this concentration. The results in table shows that use of NAA has positive effects on the growth of gardenia plant, with significant improvement in the number and length of shoots. The study indicates the importance of adjusting the concentration to achieve best growth response.

Table (4) shows effect of NAA concentrations on the emergence of plantlets from cultivated tips of shoots for gardenia plant after 12 weeks of Cultivation

Treatment BA mg/L	Percentage of Response	Shoots No. Plant Part	shoot length /cm	leaves No. Plant Part	Leaf Width/cm
0	%100	0.00	0.00	0.00	0.00
0.5	%100	2.15	1.65	1.29	0.80
1	%100	4.5	1.20	1.4	2.1
1.5	%100	10.1	0.90	6.5	1.20
2	%100	7.30	1.2	1.10	0.95
2.5	%100	11.5	0.70	7.3	1.50

L.S.D ≈ 1.81



IV. Discussion:

The table results show clear effects of benzyl adenine (BA) concentrations on the growth of Gardenia plants after 4 weeks of cultivation. All concentrations used showed complete response (100%), indicating that BA has positive effect on plant growth.

In concentration 0 mg/L, the number of shoots was 1.5 and the shoot length was 0.40 cm, indicating no growth stimulation. As the concentration increased to 0.5 mg/L, the number of shoots decreased to 1.2, but the shoot length increased to 0.87 cm, which could indicate that the lower concentration may not be sufficient to stimulate overall growth, but it may promote certain aspects of growth (17)

The concentration 1 mg/L showed best results, with the number of shoots reaching 1.9 and shoot length 11.0 cm.

In concentration 1.5 mg/L, table recorded 2.0 shoots and shoot length 0.60 cm, indicating that increasing the concentration may lead to different results. The concentration 2 mg/L showed decrease in the number of shoots to 1.1 and shoot length 0.10 cm, indicating that the higher concentration may have an inhibitory effect on growth.

Finally, concentration 2.5 mg/L was the lowest, that recorded 0.3 shoots and shoot length 0.50 cm. These results support hypothesis that high concentrations of BA may lead to negative effects on growth.

Generally, the results indicate that use of BA can be effective in promoting Cardinia plant growth, but concentration must be adjusted to achieve optimal results.

The table results show clear effects of BA concentrations on the multiplication of transplants of Gardenia plants after 12 weeks of cultivation. All concentrations used showed complete response (100%), indicating that BA has positive effect on plant growth, but the results differed significantly with regard to number of shoots.

In concentration 0 mg/L, the number of shoots was 1.00 and the shoot length was 2.05 cm, indicating no growth stimulation. With increasing the concentration to 0.5 mg/L, significant increase in the number of shoots was observed, reaching 2.25, also the number of leaves increased significantly to 5.10.

In concentration 1 mg/L, the number of shoots was 1.75 and their length 0.70 cm, indicating that concentration was not as effective compared to the concentration 0.5 mg/L. The 1.5 mg/L concentration showed the best results, with the number of shoots reaching 4.5 and the shoot length reaching 1.05 cm, reflecting the effectiveness of BA in promoting growth.

In concentration 2 mg/L, table recorded 3.00 shoots and shoot length 1.55 cm, indicating that concentration was still effective but lower than 1.5 mg/L concentration. The 2.5 mg/L concentration was the lowest, recording 0.55 shoots and shoot length 0.45 cm. This suggests that high concentration of BA may cause growth inhibition.

Generally, results indicate that use of BA can be effective in promoting multiplication of Gardenia plants., but the concentration must be adjusted to achieve the best results.

The results of the table shows clear effects of NAA (naphthalene acetic acid) concentrations on the emergence of cultivated from transplanting shoot tips for Gardenia plant after 4 weeks of cultivation, while the response at 0 mg/L was zero (0.00), all other concentrations showed a complete response (100%).

In concentration of 0.5 mg/L, number of shoots was 5.26 and the shoot length was 1.35 cm, indicating that concentration was effective in enhancing growth.

In concentration of 1 mg/L number of shoots was 2.9 and the shoot length was 1.0 cm, which indicates that concentration was not as effective compared to concentration 0.5 mg/L. However, at concentration 1.5 mg/L, significant increase in the number of shoots (8.5) and observed the shoot length (2.3 cm) supporting the effectiveness of NAA in promoting growth.

In concentration 2 mg/L, Table 4.10 recorded shoot and shoot length 1.10 cm, indicating that concentration was still effective, but lower than concentration 1.5 mg/L. The 2.5 mg/L concentration showed higher shoots number (10.3), but lower shoot length (0.50 cm). This may indicate that higher concentrations of NAA can increase the number of shoots, but have negative effects on shoots length.

Generally, the results indicate that use of NAA can be effective in promoting shoots emergence from shoots tip transplants of *Cardinia*, but concentration must be adjusted to achieve optimal results.

The table results show clear effects of naphthalene acetic acid (NAA) concentrations on emergence of shoots from shoots tips for *Gardenia* plant after 12 weeks of cultivation. All concentrations used showed complete response (100%), but the results varied significantly in terms of number of shoots, their length, and number of leaves.

In concentration 0 mg/L, the response was zero (0.00), indicating no growth. As the concentration increased to 0.5 mg/L, the number of shoots was observed to be 5.12 and the shoot length to be 1.65 cm. These results indicate that concentration was effective in promoting growth.

In concentration 1 mg/L, the number of shoots was 4.5 and the shoot length was 1.20 cm, which shows that concentration is still effective, but lower than concentration 0.5 mg/L, and observed in concentration 1.5 mg/L, significant increase in the number of shoots (10.1), which indicating the effectiveness of NAA in promoting growth.

In concentration 2 mg/L, table recorded 7.30 shoots and shoot length 2.1 cm, which indicating to effective concentration, but not as effective as concentration 1.5 mg/L. The 2.5 mg/L concentration recorded the highest number of shoots (11.5), but lower shoot length (0.70 cm). This may indicate that high concentration of NAA may cause undesirable effects on shoot length.

Generally, the results indicate that use of NAA can be effective in promoting shoots emergence from shoots tip transplants for *Cardinia* after 12 weeks, but concentration must be adjusted to achieve optimal results. Further studies are necessary to determine the optimal concentration that promotes growth without negatively impacting the plant.

V. Conclusion

The study demonstrated the success of vegetative propagation technique for propagating *Gardenia*, and it is necessary to maintain high air humidity in the early stages of acclimatization, due to the sensitivity of *Gardenia* plants to low humidity. Regarding the yield of laboratory cuttings from live plants in the laboratory, use of vegetative propagation technique, by cultivating the initial cutting, followed by replanting it multiple times, and forming propagation shoots, allows for production of approximately 22 live plants from single cutting one year after cultivating, in addition to other shoots for in vitro propagation. Active phytochemicals are group of chemical compounds that occur naturally in plants. The most important bioactive compounds analyzed in callus farms of *Gardenia jasminoides* were iridoid glucoside, guanylic acid, crocin, geniposide, and genipin. The production of these compounds in callus farms was also increased by using them as a catalyst, which was added to the growing callus culture medium, which indicates the importance of plant tissue culture technology in producing effective compounds from different plant species in reasonable quantities, within short time frame, and in restricted and controlled space..

In addition to the growth-stimulating substances such as Auxins and Cytokinins which present in seaweed extract, that work to increase growth, the amino acids and organic matter contained in the herbal extract help preserve moisture and nutrients in the soil and prepare it for the roots, which leads to an increase in the vegetative growth of plant

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