Study on the Effect of Explant Type and Benzyladenine (BA) in the Micropropagation of *Gardenia jasminoides* During the Initiation and Multiplication Stages.

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Abstract

The study was conducted to investigate the effect of explant type and benzyl adenine (BA) on the initiation and multiplication stages of Gardenia jasminoides Ellis cultured in vitro. The experiment involved using shoot tips and double stem nodes treated with three different concentrations of BA (0, 1.0, and 2.0 mg L^{-1}). A completely randomized design (CRD) was applied for both the initiation and multiplication stages, with ten replicates per experimental unit. The means were compared using the Least Significant Difference (L.S.D.) test at a probability level of 5%.

During the initiation stage, the treatment nB2 recorded the highest value for the number of leaves with an average of 9.67 leaves $plant^{-1}$. The treatment aB2 gave the highest values for both the number of shoots (2.33 shoots $plant^{-1}$) and shoot length (13.34 cm). Moreover, the treatment aB1 achieved the highest value for the percentage of successful explants, reaching 94.33%. Similarly, the treatment nB2 also recorded the highest value for the number of leaves (9.67 leaves $plant^{-1}$).

During the multiplication stage, the treatment aB2 gave the highest value for the number of leaves (22.67 leaves $plant^{-1}$). For the number of shoots, the treatment aB1 outperformed all other treatments, recording the highest value (4.667 shoots $plant^{-1}$). Regarding shoot length, the treatment nB2 achieved the highest value, reaching 25.35 cm.

Introduction

Gardenia jasminoides is an evergreen shrub belonging to the Rubiaceae family. It is a seasonal plant that flowers once per year (8). Its white flowers are fragrant and widely used as cut flowers and in garden landscaping (6,4). *Gardenia*

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jasminoides is also a popular horticultural species and has been traditionally used in folk medicine in various regions to treat numerous health conditions. Its flowers and other plant parts contain a range of bioactive compounds that have been examined in several scientific studies, including flavonoids, glycosides, alkaloids, terpenoids, and volatile compounds (15, 20,21,23).

Gardenia can be propagated sexually through seed and asexually through cuttings, grafting, and tissue culture (1,2). However, conventional propagation of gardenia often faces challenges such as susceptibility to nematode infections—one of the primary constraints affecting plant growth—and a drop in temperature below 19°C for more than two weeks can lead to reduced mineral uptake and chlorosis in leaves (5).

Plant tissue culture plays a significant role in modern agriculture, particularly in the precise propagation of plants. Micropropagation is currently one of the preferred methods for propagating woody plants due to its advantages—most notably, the ability to produce large numbers of true-to-type plants in a relatively short time and throughout the year, in addition to generating pest- and disease-free plantlets in bulk (7).

The initiation stage is the first step in plant tissue culture, where explants (such as nodes, leaves, or shoot tips) are sterilized and cultured in a nutrient-rich medium supplemented with plant growth regulators. The multiplication stage follows initiation and is crucial for producing a high number of plantlets in a short period. In this phase, the initial tissues are stimulated to form new shoots through cellular proliferation (14).

Plant Growth Regulators (PGRs) are organic compounds that influence plant physiological processes even at very low concentrations. PGRs are categorized into natural regulators (such as auxins, gibberellins, and cytokinins) and synthetic ones produced chemically. They are used in agriculture to enhance productivity—such as accelerating seed germination, controlling flowering, improving fruit quality, and reducing post-harvest losses (9).

Cytokinins play a key role in cell division, promoting root and shoot growth and increasing the number of flowers. In *Gardenia jasminoides*, cytokinins can stimulate branching and flowering. A study on gardenia reported that supplementing the medium with benzyladenine (BA) led to an increase in flower number and lateral shoot development. Cytokinins also appear effective in promoting vegetative propagation and enhancing growth rates (22).

Materials and Methods

This study was conducted in the Tissue Culture Laboratory of the Department of Horticulture and Landscape Engineering, College of Agriculture, University of Tikrit, during the 2022–2023 growing season. *Gardenia jasminoides* explants (shoot tips and nodal segments) were excised from seedlings, surface-sterilized with 6% sodium hypochlorite, and cultured on modified MS medium supplemented with different concentrations of IBA and BA.

The experiment included both the initiation and multiplication stages with three concentrations of BA, each accompanied by a fixed concentration of IBA as shown in the following table (1):

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ultiplication Stage ng L⁻ ¹	itiation Stage ng L⁻ ¹	reatment
A 0.0 BA 0.2	A 0.0 BA 0.2	
A 1.0 BA 0.2	A 1.0 BA 0.2	
A 2.0 BA 0.2	A 2.0 BA 0.2	

Experimental Factors:

- Factor A: Explant Type (a: Shoot Tip, n: Nodal Segment)
- Factor B: BA concentration (0, 1, 2 mg L⁻ ¹)

Preparation of Culture Medium
800 mL of distilled water was used for the preparation, to which 30 g L⁻ ¹ of sucrose and 4.43 g of MS powder (according to manufacturer's recommendation) were added. PPM was added at 1–2 mL L⁻ ¹ to prevent microbial contamination. Plant growth regulators were then added, the volume adjusted to 1 L, pH was set to 5.8, and 6.5 g L⁻ ¹ agar was incorporated. Culture vessels were filled with 15–20 mL of the medium, sealed, and sterilized using an autoclave (16).

Explant sterilization

Explant materials (3–4 cm in length) were collected from the field and washed several times with distilled water. They were then soaked in a fungicide solution, rinsed, and treated with a solution of ascorbic and citric acid (50 mg L⁻ ¹ and 100 mg L⁻ ¹, respectively) for 30–60 minutes. Afterward, they were dipped in 70% ethanol for 20 seconds, followed by immersion in 6% sodium hypochlorite for surface sterilization (11).

Explant Culturing

Sterile explants were transferred to the laminar airflow cabinet and sectioned into 1.5–2 cm pieces. They were then cultured on 20–30 mL of MS medium under sterile conditions. Cultures were maintained at 23–25°C with a photoperiod of 16 hours light and 8 hours darkness. Media were renewed after 30 days (10).

Studied Traits
Measurements were taken after 30 days for the initiation stage and 60 days for the multiplication stage.

Initiation Stage:

Culture Establishment Rate (%):

Success Rate=Number of Successful CulturesTotal Cultures×100\text{Success Rate} = \frac{\text{Number of Successful Cultures}}{\text{Total Cultures}} \times 100Success Rate=Total CulturesNumber of Successful Cultures×100

2. Number of Leaves
3. Number of Shoots
4. Shoot Length

Multiplication Stage:

1. Number of Leaves
2. Number of Shoots
3. Shoot Length

Results and Discussion Effect of Explant Type and Cytokinin (BA) Concentration on the Mean Number of Leaves During the Initiation Stage

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The data presented in Table 2 indicate a significant effect of explant type on the mean number of leaves. The nodal segment (n) treatment significantly outperformed the shoot tip (a) treatment, recording a mean of 9.22 leaves per plant. In contrast, the BA concentration alone did not show statistically significant differences among the values.

Regarding the interaction between explant type and cytokinin concentration, the combination of nodal segment (n) with 2.0 mg L^{-1} BA (B2) yielded the highest number of leaves at 9.67 per plant. Conversely, the lowest value was recorded for the shoot tip (a) with 0.0 mg L^{-1} BA (B0), which produced 4.33 leaves per plant.

Table 2. Effect of explant type and cytokinin (BA) concentration on the mean number of leaves during the initiation stage

ytokinin / x plant	A mg.l			verge x plant
pical	33	67	67	89
od	00	00	67	22
verge BA	67	83	17	v%= 12.5
S.D	968 ytokinin	186 x plant	677 teraction	

Effect of Explant Type and Cytokinin (BA) Concentration on the Mean Number of Shoots During the Initiation Stage

The data presented in Table 3 indicate that there were no statistically significant differences between the explant types (a: shoot tip and n:

node) in terms of the mean number of shoots. However, regarding the effect of cytokinin, treatment B2 (2.0 mg L^{-1} BA) significantly outperformed treatment B0 (0.0 mg L^{-1} BA), with the highest mean number of shoots at 2.17 shoots per plant. There was no significant difference between B2 and B1.

As for the interaction between explant type and cytokinin concentration, the combinations a × B1 and a × B2 both recorded the highest number of shoots at 2.33 shoots per plant. In contrast, the lowest value was observed in the combination a × B0, which yielded only 1.00 shoot per plant.

Table 3. Effect of explant type and cytokinin (BA) concentration on the mean number of shoots during the initiation stage

ytokinin / x plant	A mg.l^{-1}			verge x plant
pical	00	33	33	89
od	67	67	00	78
Averge BA	33	00	17	c.v%=25.7
S.D	484 Cytokinin	593 Ex plant	839 Interaction	

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Effect of Explant Type and Cytokinin (BA) Concentration on Shoot Length (cm) During the Initiation Stage

According to the data presented in Table 4, there was no statistically significant difference between the explant types (a: shoot tip and n: node) regarding mean shoot length. In terms of cytokinin concentration, treatment B1 (1.0 mg L^{-1} BA) significantly exceeded treatment B0 (0.0 mg L^{-1} BA), recording the highest mean value of 13.18 cm. However, no significant differences were observed between B1 and B2 (2.0 mg L^{-1} BA). The interaction between explant type and cytokinin showed that the combination a × B2 gave the highest shoot length, reaching 13.34 cm, while the combination a × B0 recorded the lowest value at 10.48 cm.

Table 4. Effect of explant type and cytokinin (BA) concentration on shoot length (cm) during the initiation stage

Cytokinin / x plant	A mg.l^{-1}			verge x plant
pical	0.48	3.30	3.34	2.37
od	1.16	3.06	2.66	2.29
Averge BA	0.82	3.18	3.00	c.v%=9.7
S.D	222	497	117	
	Cytokinin	Ex plant	Interaction	

Effect of Explant Type and Cytokinin (BA) Concentration on Culture Establishment Success (%) During the Initiation Stage

The data in Table 5 indicate that the explant type had a significant effect on culture establishment success. The shoot tip explant (treatment *a*) significantly outperformed the nodal segment (*n*), recording a success rate of 92.78%. As for the cytokinin factor, treatment B1 (1.0 mg L^{-1} BA) showed a higher success rate (92.17%) compared to B0 (0.0 mg L^{-1} BA), although no statistically significant difference was observed between B1 and B2 (2.0 mg L^{-1} BA). Regarding the interaction between explant type and cytokinin concentration, the combination of *a* × *B1* produced the highest success rate at 94.33%, whereas the *n* × *B0* combination recorded the lowest value at 89.33%.

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Table 5. Effect of explant type and cytokinin (BA) concentration on culture establishment success (%) during the initiation stage

ytokinin / x plant	A mg.l^{-1}			verge x plant
pical	.50	.33	.33	.78
od	.00	.00	.67	.22
verge BA	.33	.17	.50	v%=1.6
S.D	531 Cytokinin	875 x plant	652 Interaction	

Effect of Explant Type and Cytokinin (BA) Concentration on the Average Number of Leaves During the Multiplication Stage

The data presented in Table 6 show that, during the multiplication stage, the explant type had a significant effect on the number of leaves. The shoot tip explant (*a*) recorded the highest average number of leaves, reaching 16.89 leaves per plant, outperforming the nodal segment (*n*). Regarding the cytokinin factor, treatment B2 (2.0 mg L^{-1} BA) demonstrated superiority over the other concentrations, yielding the highest value of 18.83 leaves per plant.

As for the interaction between explant type and BA concentration, the combination of shoot tip (*a*) with B2 resulted in the highest number of leaves, reaching 22.67 leaves per plant. In contrast, the lowest value was observed in the *a* × *B0* interaction, which recorded only 8.00 leaves per plant.

Table 6. Effect of explant type and cytokinin (BA) concentration on the average number of leaves during the multiplication stage

Cytokinin / x plant	A mg.l^{-1}			verge x plant
pical	00	.00	.67	.89
od	.33	.33	.00	.56
Averge BA	.67	.17	.83	c.v%=11.1
S.D	729 Cytokinin	117 Ex plant	994 Interaction	

Effect of Explant Type and Cytokinin (BA) Concentration on the Average Number of Shoots During the Multiplication Stage

Table 7 illustrates that the explant type significantly affected the average number of shoots during the multiplication stage. The shoot tip explant (*a*) showed a superior performance over the nodal segment (*n*), recording the highest value of 2.778 shoots per plant. Regarding cytokinin concentrations, treatment B1 (1.0 mg L^{-1} BA) outperformed the other treatments, yielding the highest value of 3.333 shoots per plant.

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In terms of the interaction between explant type and BA concentration, the combination of shoot tip (*a*) with B1 exhibited the most significant effect, producing the highest number of shoots at 4.667 shoots per plant.

Table 7. Effect of explant type and cytokinin (BA) concentration on the average number of shoots during the multiplication stage

Cytokinin / x plant	A mg.l^{-1}			verge x plant
pical	000	667	667	778
od	000	000	667	222
Averge BA	500	333	667	c.v%=16.3
S.D	4193 Cytokinin	5136 Ex plant	7263 Interaction	

Effect of Explant Type and Cytokinin (BA) Concentration on the Average Shoot Length During the Multiplication Stage

Table 8 shows that the explant type significantly influenced the average shoot length during the multiplication stage. The nodal segment explant (*n*) outperformed the shoot tip (*a*), recording the highest shoot length of 19.82 cm. Regarding cytokinin concentrations, treatment B2 (2.0 mg L^{-1} BA) resulted in the highest average shoot length of 23.67 cm.

The interaction between explant type and BA concentration revealed that the combination of the nodal segment (*n*) with B2 exhibited the highest shoot length among all treatments, reaching 25.35 cm.

Table 8. Effect of explant type and cytokinin (BA) concentration on the average shoot length during the multiplication stage

ytokinin / x plant	A mg.l^{-1}			verge x plant
pical	3.18	0.15	1.99	3.44
od	3.26	0.87	5.35	0.82
verge BA	3.22	0.51	3.67	c.v%=5.5
S.D	062 Cytokinin	300 Ex plant	839 Interaction	

Discussion

The superiority of the nodal explant (*n*) over the shoot tip explant (*a*) in terms of the average number of leaves and shoots may be attributed to the direct influence of the nodal segment on rooting efficiency and overall growth performance in tissue culture. This could be explained by various

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physiological and morphological factors, including the cellular structure and endogenous distribution of plant hormones within these tissues. Nodal explants contain latent axillary buds

surrounded by active meristematic cells, which are physiologically predisposed to divide and grow under optimal in vitro conditions.

Cytokinins are known to stimulate cell division and the formation of lateral shoots, as noted by (19). The significant interaction observed between the *n* explant and B1 treatment may be due to the high division potential of these cells and their

pronounced responsiveness to plant growth regulators,

particularly cytokinins such as BA. This responsiveness enhances the rapid and efficient activation of lateral buds, unlike shoot tips, which often contain higher levels of growth-inhibiting hormones such as auxins, thus limiting lateral branching. These findings are consistent with those of (18).

The observed superiority of treatment B1 in shoot length may be attributed to the role of cytokinins in promoting cell wall expansion through the activation of enzymes like expansins, allowing cells to absorb water and elongate. Cytokinins also promote longitudinal shoot growth when applied at optimal concentrations, especially during early developmental stages, (17).

During the multiplication stage, the enhanced performance of shoot tips in terms of leaf and shoot number may be attributed to their content of apical meristematic cells with high division and growth capacity. This active tissue continuously generates new cells and produces endogenous growth hormones—primarily auxins and cytokinins—that stimulate leaf and shoot formation under suitable conditions. Additionally, the shoot apices exhibit the highest mitotic activity in the plant, making them an excellent source for rapid and prolific growth in vitro.

Furthermore, shoot tips are particularly responsive to BA during the early stages of development. When apical dominance is disrupted by growth regulators, lateral shoots are extensively initiated, and leaf number increases due to rapid cell division in the apical tissue. This observation is in agreement with (12,13).

The observed superiority of nodal explants in shoot length during the multiplication stage may be attributed to the presence of axillary buds located at the leaf axils, which are typically dormant in the parent plant. However, once activated by growth regulators in the culture medium, these buds exhibit a high elongation potential. Moreover, nodal segments have a relatively larger exposed surface area post-inoculation, enhancing the absorption of nutrients and growth regulators from the culture medium and thus supporting elongation. The significant interaction between *n* explants and BA treatment could be explained by the fact that once dormant buds receive cytokinin signals, they rapidly initiate growth and produce relatively longer shoots due to reduced apical dominance compared to shoot tips. (3)

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