"Improving Shoot or Nodal Multiplication, and In Vitro Rooting of *Gardenia jasminoides* Using Amino Acids as Biostimulants"

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Abstract

This study was conducted in the plant cell and tissue culture laboratory of department of horticulture and landscape design university of Mosul during the period from 2024 to 2026, the investigation done to explain the role of methionine and glutamine at (0.0, 50, 100, 150, 200) with 2.0 mg L^{-1} BA on gardenia shoot tips or nodes multiplication and rooting shoots produced *in vitro* using IBA at (0.0, 0.5, 1.0, 1.5, 2.0) mg L^{-1} in MS media: data refers that nodes multiplication was better compared with shoot tips, the results also showed that methionine treatments showed numerically higher values than glutamine in nodes or shoots proliferation, culture nodal segments on a medium with 200 mg L^{-1} methionine resulted in the highest average number of shoots (5.2 shoots per explant), whereas culturing shoot tips at 50 mg L^{-1} methionine produced the highest average number of shoots (3.6 shoots per explant) after eight weeks, but culturing both shoots and nodal segments on a medium supplemented with 50 mg L^{-1} glutamine resulted in the highest multiplication (3.2 shoots per explant) after eight weeks of culture for both explant types, culture shoots produced *in vitro* in MS media contain 2.0 mg L^{-1} IBA give the highest root percentage, roots number, longest root, 100%, 5.1, 1.05 cm respectively after four weeks from culture, all plantlets produced from these experiments were acclimatized and transferred to field Successfully.

Keywords: Gardenia, Proliferation, Glutamine, Methionine.

Introduction

Gardenia (*Gardenia jasminoides*) is an evergreen shrub belonging to the coffee family Rubiaceae. It is native to China, and numerous hybrids have been developed from it, including the cultivar Veitchii, which is the subject of this study. Other species of Gardenia include *Gardenia thunbergia*, which is native to South Africa and is commonly use as a rootstock for grafting [14]. The height of Gardenia shrubs ranges from 1-2 meters, bearing white flowers with a fragrant aroma [19,5, 4,13]. The plant requires indirect light, and optimal growth occurs under moderate temperatures ranging between 15-25 °C. It prefers slightly acidic soils with a pH of 5-5.5 that are rich in organic matter [19,14] Gardenia is considered an ornamental shrub widely used in home and public gardens, its flowers are also used for cutting and perfume extraction. The plant is characterized by the presence of certain bioactive compounds such as rutin and crocin. It is propagate vegetatively through grafting or cuttings [4,9]. Amino acids are the fundamental building blocks of proteins and play a central role in plant responses to environmental stresses and in enhancing growth, their function is not limited to protein synthesis but also extends to regulatory roles, such as the biosynthesis of enzymes that catalyze all biochemical reactions within the cell, they are involved in osmotic regulation, stress resistance, and hormone synthesis, and

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they promote photosynthesis and stimulate root growth. Additionally, amino acids have the ability to chelate micronutrients such as iron and zinc, facilitating their absorption and translocation within the cell [12,15]. Cytokinins play a major role in the proliferation of shoots and nodes in many plants, Previous studies indicated that cultivating gardenia shoot tips on MS medium enriched with 2.0 mg L^{-1} BA yielded 2.69 shoots $explant^{-1}$ after eight weeks, [16] found that incubation shoot tips on MS medium contain 3 mg L^{-1} BA with 0.5 mg L^{-1} IAA produced 2.8 shoots per explant, [1] reported that cultivating gardenia shoot tips on MS medium enriched with 2.0 mg L^{-1} BA yielded 2.69 shoots $explant^{-1}$, [2] declare that inoculate shoot tips on MS medium enriched with different concentrations of BA resulted in the highest shoot number 8 shoots per explant at 2 mg L^{-1} after eight weeks. [16] also indicated that culturing nodal segments on MS medium with 3.0 mg L^{-1} BA produced 2.7 shoots per explant after eight weeks, [2] say that cultivating nodal segments on MS medium with 2 mg L^{-1} BA produced the highest number of shoots 5 shoots per explant after eight weeks and that regenerated shoots rooted at a rate of 90% with an average of 5 roots per explant and an average root length of 0.56 cm when cultured on MS medium supplemented with 0.5 mg L^{-1} IBA after four weeks.

Most plants are capable of synthesizing their essential amino acid requirements necessary for cell proliferation and regeneration. However, the external supply of amino acids to the culture medium plays an important role in stimulating cell growth and organ formation [6]. [8] achieved a significant increase in the number of shoots from apical meristem culture of strawberry (*Fragaria* × *ananassa* Duch. cv. Chandler) grown on MS medium supplemented with 25 and 50 mg L^{-1} of the amino acid glutamine, the number of shoots reached 5.0 shoots per explant for both treatments, with shoot lengths of 1.0 and 0.83 cm, respectively, after 8 weeks of culture. [17] reported that the addition of glutamine at concentrations ranging from 10-100 mg L^{-1} to the culture medium significantly increased the multiplication rate of *Cordyline fruticosa* Similarly, a concentration of 100 mg L^{-1} glutamine produced the same effect in *Gerbera jamesonii*. However, the addition of glutamine showed no significant effect on shoot length or on the number and length of roots in the studied species. [10] obtained the highest shoot growth from nodal explants of *Hibiscus moscheutos* derived from tissue culture, consisting of two nodes with the leaves removed from the lower node. Shoots grown on MS medium supplemented with 10 mg L^{-1} glutamine showed enhanced growth, with the longest shoot reaching 5.8 cm, which was significantly superior to the other treatments after 7 weeks of culture.

This study aims to investigate the role of glutamine and methionine in the multiplication of nodes and shoot tips of gardenia and subsequently in the rooting of the regenerated shoots.

Materials and Methods

This study was conducted in the Plant Tissue Culture Laboratory, Department of Horticulture and Landscape Gardening, College of Agriculture and Forestry, University of Mosul, from August 2024 to February 2026, where shoot tips and nodal segments of *Gardenia jasminoides* cv. Veitchii were collected from 15-year-old shrubs grown in a private house in Iraq, Mosul city, all leaves were removed and the explants were washed under running tap water for 10 minutes then surface sterilized using commercial sodium hypochlorite (Clorox) solution at a ratio of 1:10 v/v for 20 minutes with addition of

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Tween-20 followed by three rinses with sterile distilled water for 3-5 minutes each [3], the explants were cut into approximately 1 cm segments and cultured on MS medium supplemented separately with the amino acids glutamine and methionine at concentrations of 0, 50, 100, 150, and 200 mg L^{-1} with all treatments containing 2 mg L^{-1} BA; the shoots produced from multiplication were transferred to media supplemented with IBA at concentrations of 0.0, 0.5, 1.0, 1.5, and 2.0 mg L^{-1} for rooting; all cultures were maintained in a growth room at 25 ± 2°C, under a light intensity of 3000 lux and a photoperiod of 16 hours light and 8 hours dark provided by white fluorescent lamps. Well-growing plantlets were removed and their roots were washed with water to eliminate the culture medium. They were then transplanted into sterilized peat moss and covered with glass covers, placed in growth room, after two weeks they were transferred to preparations room and removing the glass covers gradually, after another two weeks the covers were completely remove then remain in the laboratory for three additional weeks before being transferred to the plastic house. The experiment was arranged in a completely randomized design (CRD) and means were compared using Duncan's Multiple Range Test at a 5% probability level [7], with each treatment consisting of 10 replicates and each replicate containing one explant.

Results and Discussion

Table (1) indicates that methionine significantly affected the multiplication of shoot tips of *Gardenia jasminoides*, as all methionine treatments surpassed the control in the average number of shoots during the initiation stage; data from the multiplication stage showed that the 50 mg L^{-1} methionine treatment produced the highest mean number of shoots 3.60, significantly exceeding the control (Fig. 1), and this treatment also resulted in the greatest shoot length (2.65 cm) and highest number of leaves (12.80 leaves).

Table 1. Effect of methionine in initiation and multiplication of *Gardenia jasminiodes* shoots tips culture on MS media.

Methionine mg L^{-1}	Establishment stage (after 4 week)				Multiplication stage (after 8 week)			
	Response %	Shoots number	Shoot length	Leaves number	Response %	Shoots number	Shoot length	Leaves number
0.0	100 %a	1.20 b	1.10 c	3.20 c	100 %a	1.60 b	1.55 b	7.10 b
50	100 %a	2.10 a	1.65 b	5.50 a	100 %a	3.60 a	2.65 a	12.80 a
100	100 %a	2.10 a	2.10 a	4.60 ab	100 %a	3.40 a	2.75 a	13.60 a
150	100 %a	2.10 a	1.85 ab	3.60 bc	100 %a	2.80 a	3.15 a	8.30 b
200	100 %a	1.80 a	2.10 a	4.00 bc	100 %a	2.50 ab	2.70 a	8.50 b

* values followed by the same letters within each column are not significantly different according to Duncan's Multiple Range Test at the 5% probability level.

* all treatments were supplemented with 2 mg/L BA.

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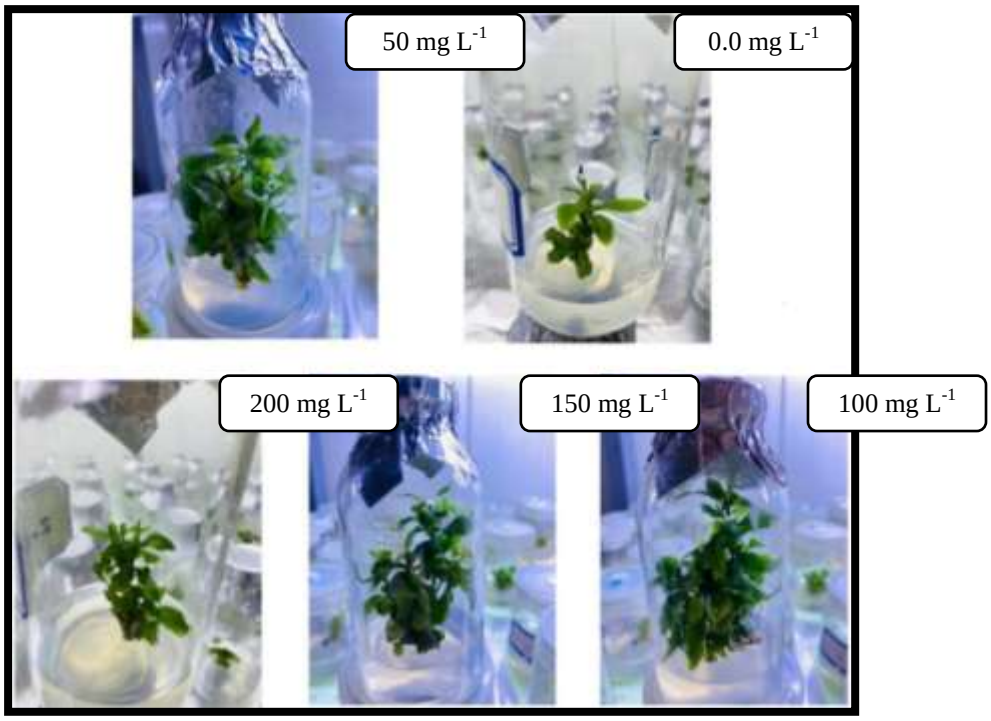

Figure 1. Effect of methionine on the shoot tips of Gardenia grown in MS medium after 8 weeks of cultivation.

It is evident from Table (2) that methionine has a significant effect on the multiplication of gardenia plant nodes, as all methionine treatments significantly outperformed the control treatment in the number of shoots at initiation stage. From the methionine results for the multiplication stage, Table (2) shows that the 200 mg L^{-1} methionine treatment produced the highest mean number of shoots, reaching 5.20 shoot per plant explant (Fig. 2) with a significant difference compared with all other treatments, this treatment also achieved the highest mean shoot length 3.00 cm, as well as the highest mean number of leaves 15.90 leaves per explant.

Table 2. Effect of methionine in initiation and multiplication of *Gardenia jasminiodes* nodes culture on MS media.

Methionine mg L^{-1}	Establishment stage (after 4 week)				Multiplication stage (after 8 week)			
	Response %	Shoots number	Shoot length	Leaves number	Response %	Shoots number	Shoot length	Leaves number
0.0	100 %a	1.80 b	1.00 c	4.60 c	100 %a	2.10 c	1.80 b	8.60 b
50	100 %a	2.20 a	1.15 bc	7.60 ab	100 %a	2.60 bc	2.85 a	13.80 a
100	100 %a	2.30 a	1.45 a	7.00 b	100 %a	2.80bc	2.70 a	14.80 a
150	100 %a	2.40 a	1.55 a	8.40 a	100 %a	3.60 b	2.75 a	15.60 a
200	100 %a	2.70 a	1.40 ab	8.60 a	100 %a	5.20 a	3.00 a	15.90 a

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* all treatments were supplemented with 2 mg/L BA.

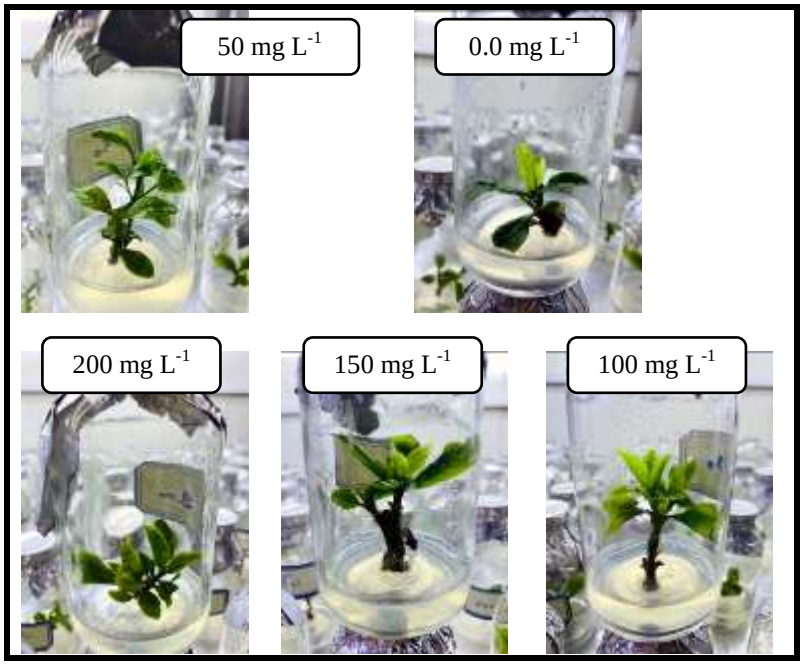

Figure 2. Effect of methionine on node multiplication of Gardenia grown on MS medium after 8 weeks of culture.

Table (3) shows that treatment with 50 mg L^{-1} glutamine achieved the highest average number of shoots 2.60 shoots per explant at the initiation stage, after sub culturing for another four weeks (multiplication stage), the same treatment also recorded the highest average number of shoots (3.20 shoots per explant), this was significantly higher than the control treatment, which produced 2.60 shoots per explant (Fig. 3). Furthermore, the 50 mg L^{-1} treatment resulted in the greatest shoot length 2.60 cm and the highest average number of leaves 12.50 leaves per explant.

Table 3. Effect of glutamine on the initiation and multiplication of *Gardenia jasminiodes* shoots tips culture on MS medium.

Glutamine mmg L^{-1}	Establishment stage (after 4 week)				Multiplication stage (after 8 week)			
	Response %	Shoots number	Shoot length	Leaves number	Response %	Shoots number	Shoot length	Leaves number
0.0	100 %a	1.90 b	1.45 bc	3.80 ab	100 %a	2.60 b	1.85 b	12.40 a
50	100 %a	2.60 a	1.80 ab	4.60 a	100 %a	3.20 a	2.15 ab	12.50 a
100	100 %a	2.30 ab	2.20 a	3.10 ab	100 %a	2.80 ab	2.60 a	9.80 b
150	100 %a	2.20 ab	1.20 c	2.80 b	100 %a	2.70 ab	2.00 b	8.60 b
200	100 %a	2.00 ab	1.55 bc	3.10 ab	100 %a	2.10 b	2.30 ab	9.20 b

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* all treatments were supplemented with 2 mg/L BA.

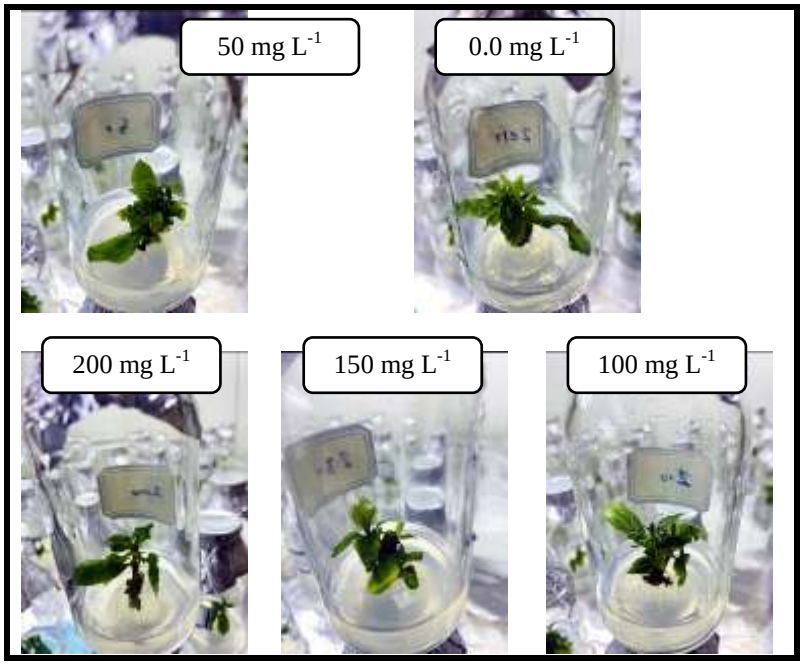

Figure 3. Effect of glutamine on the shoot tip of Gardenia grown in MS medium after 8 weeks of cultivation.

Table (4) shows that treatment with 50 mg L^{-1} glutamine produced the highest average number of shoots 2.40 shoots per explant achieved from nodal cultures grown on MS medium contain 50 mg L^{-1} glutamine, with a significant difference compared to the control treatment after 4 weeks of culture (initiation stage). After sub culturing the explants for another 4 weeks (multiplication stage), the same treatment recorded the highest average number of shoots 3.20 shoots per explant (Fig. 4) which significantly outperforming the control, It also resulted in the greatest shoot length 2.60 cm and an average of 9.50 leaves per explant.

Table 4. Effect of glutamine in initiation and multiplication of *Gardenia jasminiodes* nodes culture on MS media.

Glutamine mmg L^{-1}	Establishment stage (after 4 week)				Multiplication stage (after 8 week)			
	Response %	Shoots number	Shoot length	Leaves number	Response %	Shoots number	Shoot length	Leaves number
0.0	100 %a	1.80 bc	1.05 b	6.40 a	100 %a	2.10 b	1.30 b	10.60 a
50	100 %a	2.40 a	1.00 b	6.80 a	100 %a	3.20 a	2.60 a	9.50 a
100	100 %a	2.30 ab	1.05 b	4.90 a	100 %a	2.80 a	2.30 a	9.30 a
150	100 %a	2.00 ac	1.00 b	5.80 a	100 %a	2.70 a	2.15 a	11.00 a
200	100 %a	1.50 c	1.25 a	4.90 a	100 %a	1.60 b	2.00 a	8.50 a

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* all treatments were supplemented with 2 mg/L BA.

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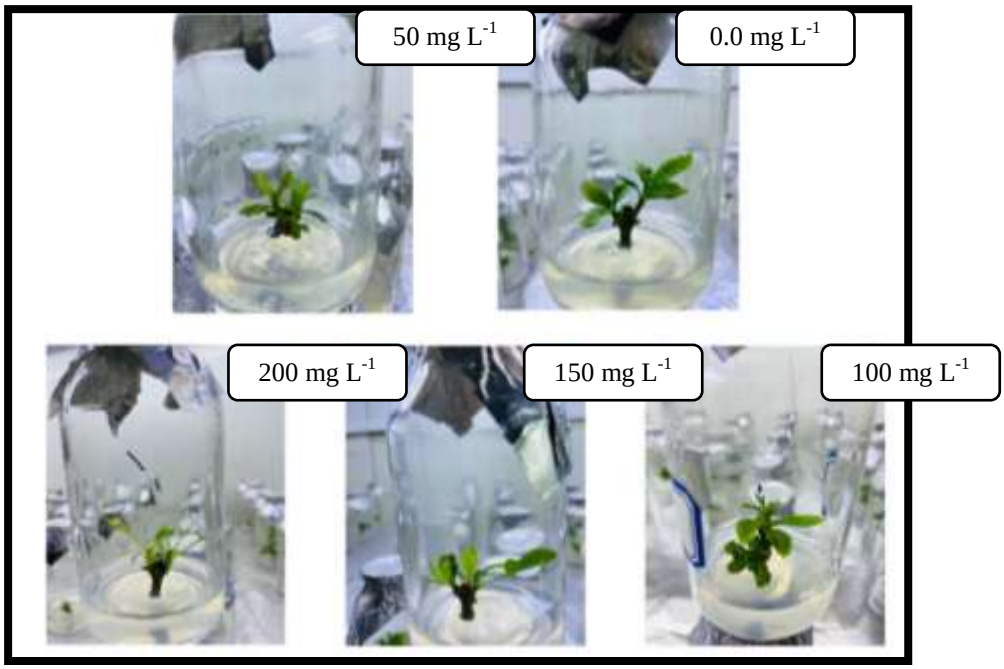

Figure 4. Effect of glutamine on Gardenia nodes multiplication after 8 weeks on MS medium.

The results of explant multiplication presented in Tables (1, 2, 3, and 4) can be explained on the basis that BA is a cytokinin that plays a role in controlling apical dominance by suppressing it, thereby increasing the number of shoots. As a result of achieving a balance between endogenous hormones and the added growth regulators, the highest values were obtained however increasing the concentration leads to a reduction in the number of shoots, as its effect becomes inhibitory [18]. The response of shoots and nodes multiplication to different amino acid treatments may be attributed to their role in providing organic nitrogen, which significantly enhances vegetative growth. In addition, amino acids are key precursors in the biosynthesis of certain plant growth-promoting hormones, some of them also help plants tolerate stress and play an important role in protein synthesis, which is essential for enzyme formation the main drivers of metabolic processes in plants [15].

Table (5) declare that all IBA treatments significantly outperformed the control in terms of rooting percentage. The highest rooting percentage (100%) was achieved at a concentration of 2.0 mg L^{-1}, and this treatment also resulted in the highest average number of roots, the longest roots, and the greatest shoot length, recording 5.10 roots, 1.05 cm, and 1.70 cm, respectively (Fig. 5-A,B) these results can be explained by the role of IBA as one of the hormones used for rooting due to its effect on stimulating cell division at the cut site leading to roots formation [11].

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Table 5. Effect of IBA on rooting of shoot tips of Gardenia culture on MS medium after four weeks.

IBA mg L□¹	Rooting (%)	Number roots	Length root	Length of the longest branch (cm)	Number of leaves	Plant height (cm)
0.0	0.0 c	0.0 c	0.0 c	1.15 b c	2.70 a	1.00 c
0.5	90 b	2.40 b	0.45 b	0.95 c	2.60 a	1.20 c
1.0	90 b	2.50 b	0.45 b	1.10 b c	2.50 a	1.30 c
1.5	90 b	3.6 ab	0.95 a	1.15 b c	2.10 a	2.00 b
2.0	100 a	5.10 a	1.05 a	1.70 a	2.50 a	3.30 a

*values followed by the same letters within each column are not significantly different according to Duncan's Multiple Range Test at the 5% probability level.

The plantlets produce from previous experiments were acclimatized under laboratory conditions in growing room for 15-20 days as they were covered with glass containers as in (Fig. 5-C), then the glass covers were removed from them and left for 15 days and then transferred to the preparation room after 15-20 days transferred to plastic house, then to the lath house, and then to the field (Fig. 5-D) with a survival rate of 100%.

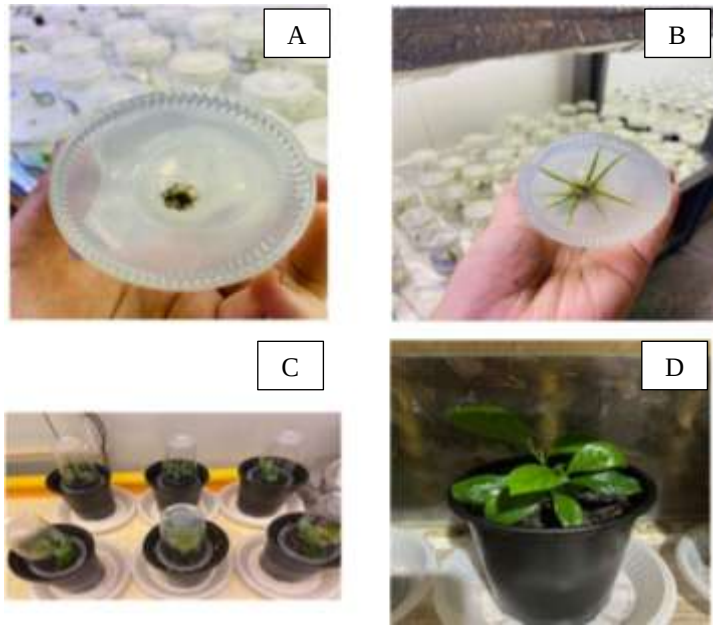

Figure 5. Rooting shoots (A- Control), (B- 2.0 mg L IBA), (C- in growing room), (D- in the field).

Conclusion

According to this study can be conclude that the amino acid methionine can be added to the MS culture medium due to its role in enhancing the proliferation of shoots and nodes of Gardenia plants, followed by rooting of the resulting shoots, their acclimatization, and subsequent transfer to the field.

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