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IMPACT OF PLANT GROWTH REGULATORS AND ADENINE SULFATE ON *GARDENIA JASMINOIDES* MICROPROPAGATION

Zaid Khaleel Kadhim[1], Makki Noaman Nayyef[2], Hasanain Ali Abdulhussein Awadh[3], Hasan Mousa Jaafar[4] and Muslim Abd Ali Abdulhussein[5]

[1] Horticulture and Landscape Depart. College of Agriculture, University of Karbala, Iraq
[2] Horticulture and Landscape Depart. College of Agriculture, University of AL-Qasim Green, Iraq
[3]Administrator of Babylon Education, Occupational Instruction, Ministry of Education, Iraq
[4]Animal production Depart. College of Agriculture, University of Karbala, Iraq
[5]Horticulture and Landscape Depart. College of Agriculture, University of Kufa, Iraq
Corresponding Author: Muslim Abd Ali Abdulhussein
E-mail : muslim.alrubaye@uokufa.edu.iq

Abstract

This study was conducted in the Tissue Culture laboratory of the Horticultural Department of the Faculty of Agriculture at Kufa University to investigate the effects of various growth regulators such as BA in (0, 0.25, 0.50, 0.75 and 1 mg L^{-1}) for the Proliferation, adenine sulfate a (0, 40, 60 and 80 mg/l) for the multiplication of herds and IBA auxins at (0, 0.50, 1.00 and 1.5 mg.l^{-1}) for shoot rooting when used in growth media for gardenia micropropagation by single node. Results were showed there are a possibility of bud growth from single node by culturing in MS media with BA at 0.75 and 1 mg. L^{-1} which achieved 100% responded percentage for bud development. The addition of adenine sulfate in various concentrations significantly increased the number and of the buds formed. When the adenine sulfate was prepared in a concentration of 80 mg.L^{-1}. 3.5 bud or shoots with 1.58 cm were formed has been added. The results showed that they had the maximum root percentage and number of roots as well as the greatest root length in media MS with 1.5 mg.l^{-1} IBA compared to the control from various concentrations of IBA.

***Keywords*:** Plant Growth Regulators, Adenine sulphates, *Gardenia jasminoides*

Introduction

Gardenia jasminoides Ellis belong to the Rubiaceae family its native was tropical and subtropical area specifically in China and Japan, and recently (Kobayashi and Kaufma, 2006; Xiao *et al*., 2017). Gardenia was first introduced to Iraq in 1959 (Tawagin, 1987). It is one of the beautiful shrubs, its flowers can be used for extracting perfumes and some chemicals substances such as phenols, quinine, carotenoids, etc., which are used as medicines, antioxidants, and microorganisms against disease (Lee *et al*., 2009). Gardenia is propagated vegetative, because its propagation sexually (with seeds) gives genetically differentiated plants, therefore it propagated vegetatively by grafting on rootstock that are tolerant of iron deficiency or by stem cutting (Al-Sultan *et al*., 1992). Plant tissue culture play an important role in agriculture field, especially in plant micropropagation, as the proliferation of tissue culture is one of the methods currently used in the propagation of woody plants because of the advantages of this method, perhaps the most important of which is to obtain On large numbers of true-type plant in a relatively short time and at any time of the year as well as the possibility of producing free–plant from infection by various insect and pathological pests in large numbers (Chavan *et al*., 2014). Cytokinins have been widely used in micropropagation of plant to encourage the number of shoots in them, and many experiments have shown the role of cytokinines in the multiplication of Gardenia in vitro. Rasheed and Duhoky (2009) indicated that the ideal concentration of BA is 5 m.g. L^{-1} stimulating growth of shoots. While Al-Noah (2009) noticed that adding BA to the nutrient media with concentrations of 0.5 to 2.5 gave the highest response. Also, Gaber and Barakat (2019) notice that 2 mg. L^{-1} BA in shoots multiplication media achieved highest shoots multiplication. Adenine sulfate is usually added to the media for as a source of the adenine nitrogenous base, and adenine sulfate has been added to the multiplication media for micropropagation of many plants, including bananas (Giap *et al*., 2012) and Date palm (Jain *et al*., 2011), as adding them increases the rate of shoots multiplication.

Auxins play a role in growth and development of roots in most plant species, and the most widely used Auxins in plant tissue culture media for stimulating root formation are NAA, IAA, IBA and (George *et al*., 2008). Al-Noah (2009) stated that IBA mediated concentration of 1 mg.L^{-1} was appropriate for rooting multiple shoots in Gardenia and shared the same result (Salim and Hamza 2017). The study aimed to employ the technology of tissue culture to multiply the Gardenia plant through studying the extent of response of the single cutting stem to multiplication on nutrient media equipped with diverse concentrations from growth plant regulators and sulfate minerals into obtain high levels of multiplication of the vegetative parts, and then study the possibility of Gardenia shoot rooting.

Materials and Methods

The study was performed in 2018-2019 in the tissue culture laboratory of the department for horticulture and landscaping, Faculty of Agriculture, University of Kufa. MS media produced by Himedia Company were used in all experiments implemented in the study. With the addition of 30 g.L^{-1} sucrose and growth regulators (according to the micropropagation stages) and 250 mg. $L^{-1}$from activated coal. The pH of the medium was adjusted to 5.7 ± 0.1 and the hardening substance of the medium was added in the form of agar in 7 g. L^{-1} then distributed uniformly in the culture vessels and autoclaved under 1.04 kg / cm^2 pressure and at

121 °C for 20 minutes .collected shoots new in length (10) cm were from

Culture establishment

Explant preparation and sterelization

Collected shoots new in length 10 cm were potted gardenia plant, then all the leaves were removed from them and washed with soap and water and then cut into a length of 2.0 to 2.5 cm to make each piece contains on one node (Figure 1) and then washed with running water for a period of 20-30 minute and were taken to the laminar air flow cabinet to beginning her surface sterilization process.

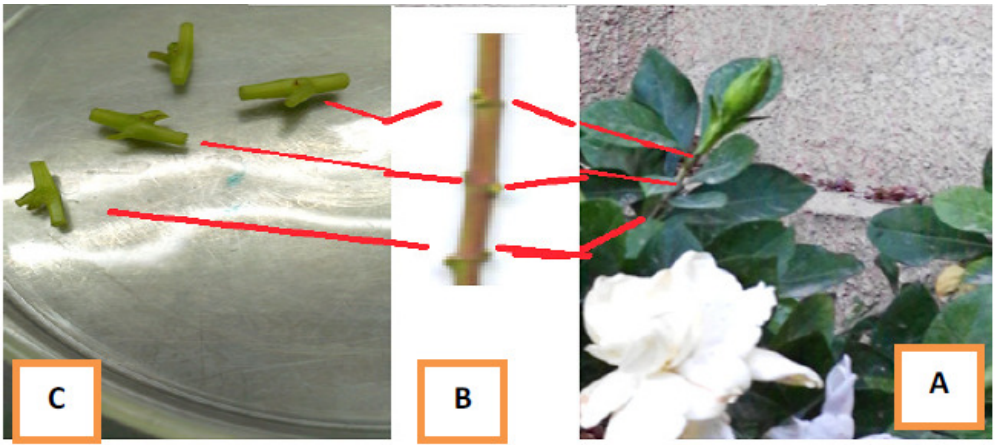

Fig. 1 : Preparing the vegetable portion used for the cultivation of plantlets
A-full plant B- new shoot after removing leaves C- Single Nodes

Nodes were sterilized with the aim of determining the suitable duration of sterilization with sodium hypochlorite (as Clorox 6%) for a period of (0, 7, 10, 15 and 20) minutes, after wards the nodes were washed with water sterile distilled 3 times to ensure the remaining sterile material was removed.

The effect of cytokinine BA

The effect of adding BA was tested with concentration (0, 0.25, 0.50, 0.75 and 1.00) mg/l in the response of single stem nodes cultivated in the MS media equipped with these concentrations. Incubate the cultures at a temperature of 25 ± 1ºC under 1000 lux to period 16 hours light then in darkness by 8 hours in growth room for 3 weeks. The response % (the percentage of growing buds) was estimated according to the following formula.

$$\text{Response}\% = \frac{\text{Number of buds grown}}{\text{Total number of cultivated buds}} \times 100$$

Shoot Multiplication Stage

Effect of adenine sulfate

Effect of various concentrations from Adenine sulfate on the multiplication and growth of shoot were studied. Adenine sulfate at (0, 40, 60 or 80) m.g. L^{-1} concentrations were added to the media MS completed with 4.00 m.g.L^{-1}BA. shoots resulting from the best treatment from establishment to transferred to MS media for stimulating the process of multiplication of the shoots, as it was cultured by one shoot (1.5 cm) for each culture vessel with ten replications per treatment and incubated at a temperature of 25 ºC ± 1 under 1000 lux for a period at 16 hours light, keep track of by 8 hours of darkness in growth room. The results were taken in terms of length shoots and number shoots after 4 weeks of incubation.

Rooting stage

The effect of adding IBA at 0, 0.5, 1 or 1.5 mg/l concentration to the 1/2MS media on percentage of rooted shoots, number and length of roots was studied. Shoots resulting from the best treatment with length 2-3 cm separate and cultured on 1/2MS media(one shoot per) with 20 replicates per treatment and incubated at a temperature of 25 ºC ± 1 under 1000 lux for a period of 16 Hours of light than by 8 hours of darkness. Results were taken in percentage of rooted shoots, length and number of roots after 4 weeks.

Statistical analysis and experimental design

All former experiments carried out by following Completely Randomized Design (CRD) with ten replicates per treatment for all experiments. Data were analyzed according to the statistical analysis program (GenStat, 2012). The means were Compared using less significant difference test (LSD) at 0.05 probability.

Results and Discussion

The establishment stage

Table (1) shows the effect of immersion durations with sodium hypochlorite solution used to sterilize single nodes and their pollution percentage. As it is noticed that the pollution (%) decrease with an increase in the duration of immersion, as the percentage of pollution reached 100% in the non-sterile vegetative parts. It was also observed that periods 7 and 10 minutes gave pollution rates of 40 and 20%, respectively, while the percentage of pollution decreased by increasing concentrations of hypochlorite Sodium where it was zero, at the duration of 20 minutes, but this treatment caused the death of all sterilized nodes.

Table 1 : Effect of the period immersion of node explants in sodium hypochlorite on the contamination percentage of cultures.

Duration (minut)	Pollution rate (%)	Notes
0	100	All vegetative parts die after being contaminated.
7 minutes	60	Natural growth of non-polluted plant parts
10 minutes	20	Good plant growth
15 minutes	10	70% of the non-polluted parts were severely damaged.
20 minutes	0	death and burning of all cultivated plant parts

Effect Benzyladenine BA

Results in Table (2) showed this BA concentrations have a significant effect on response (%) as the stem nodes grown on MS media equipped with 0.75 and 1.00 mg.l^{-1} BA gave the elevated response rate of 100% (Figures-2 A and B) which differed significantly from BA-free nutrient media which achieved lowest response in buds grown on MS-free BA media (20%) (Figure 5-C).

Table 2 : Effect of BA on the response rate of the single stem node cultured on MS media

Concentrations of BA(mg . L^{-1})	(%) Response
0	20
0.25	60
0.5	90
0.75	100
1	100
LSD 0.05	26.82

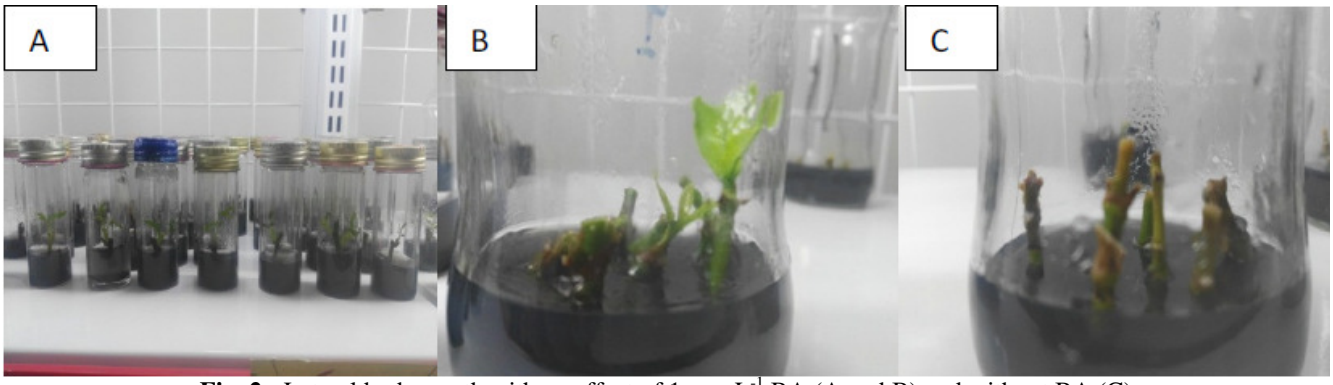

Fig. 2 : Lateral bud growth with an effect of 1 mg. L^{-1} BA (A and B) and without BA (C)

The reason for the buds response to add BA to the nutrient media may be due to the role of cytokinines at this stage in encouraging buds to grow towards the formation of shoots by balancing with the internal Auxins produced by the bud itself. These results are consistent with Al-Noah (2009), Duhoky and Rasheed, (2009), and Kozak (2011) in the necessity of adding BA in the stage of establishment culture of gardenia.

Shoots Multiplication Stage

Effect of adenine sulfate

Results in Table (3) indicate that the levels of adenine sulfate had a significant effect on the average number of shoots and their lengths, as the media provided with 80 mg.l^{-1} achieved the elevated average number of shoots (Fig. 3) and reached 3.50 shoots while media contained 0.00 mg.l^{-1} was given the lowest average number of shoots that was 1.0 shoots. The reason is due to the role of adenine sulfate similar to cytokinines in overcome the apical dominance and encourage lateral buds growth and thus increasing the number of lateral shoots resulting from single shoot.

The results also show in the same table that concentrations of adenine sulfate had a significant effect on the shoot length and the highest average length of shoots was 2.70 cm for shoots planted in MS media with 60 mg.l^{-1}, while the lowest average length of shoots was 0.55 cm in free adenine sulfate -MS medium. In the same table, a decrease in the length of the shoots is observed with an increase in the sulfate concentration of 60 mg.l^{-1}. The reason may be due to the increase in the number of shoots of this treatment and then the competition between them, which reduces the average length of the shoot.

Table 3 : Effect of adenine sulfate on the shoot number and shoots length after 4 weeks

Adenine sulfate (mg.L^{-1})	Shoots number	Shoots length (cm)
0	1.0	0.55
40	1.2	1.62
60	2.85	2.7
80	3.5	1.58
LSD 0.05	1.87	0.0661

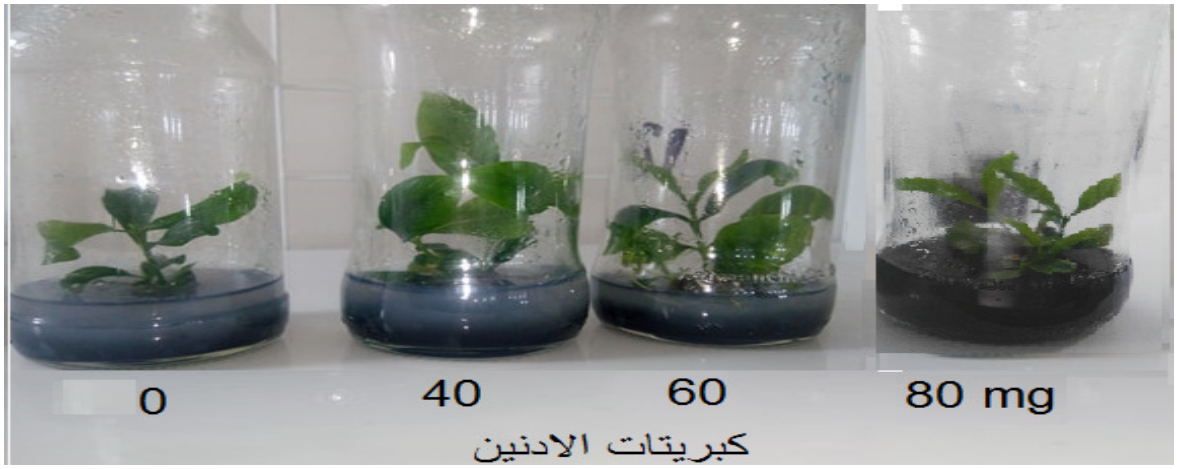

Fig. 3 : Effect of adenine sulfate on the average number shoots and length after 4 weeks planting stem nodes.

Rooting stage

Results in Table (4) showed that adding IBA with different concentrations to the MS medium had a significant effect on rooting percentage for the Gardenia branch, as the Percentage increased by increasing the added concentration to the media to the highest value within a concentration of 1.5 mg.l^{-1}, which achieved 90% while the comparison treatment was recorded 10 %, The results also indicate in the same table that there were significant differences between the concentrations in the average number of root, as the two concentrations exceeded 1 and 1.5 mg significantly over the other concentrations, as the average number of roots in the concentration was 1.0 mg/l and 1.5 mg.l^{-1} (2.1 and 3 roots) (Figure 4), while the average number of roots in the comparison was 1 root / branch. Concentration at 1.0 mg.L^{-1} give on average root length of 5.6 cm versus 1 cm for the comparison treatment, IBA are The most commonly used auxins for root trunk cuts and root tissue cultures have produced microcuts. It has been repeatedly confirmed that auxin is necessary for the emergence of random roots in the stems, and in fact it has been shown that the divisions of the original cells of the first root depend on endogenous or applied cells auxin (Hartmann *et al*., 2014). These results are consistent with Salim, and Hamza, (2017), Al-Noah (2009), Rasheed and Duhoky (2009), (Kozak, 2011), (Kadhim *et al*., 2019) when using the IBA at a concentration of 1.5 mg.l^{-1} as it gave the highest rate of number, length of roots and rooting percentage.

Table 4 : Effect of IBA concentration on Percentage of Gardenia shoot Rooting in MS Medium

Concentrations of IBA mg / L	Rooting percentage (%)	Average number root	Average length of roots cm
صـــفر	10	1	2
0.5	30	1.2	3
1	70	2.1	5.6
1.5	90	3	4.9
LSD	20	0.8	1.2

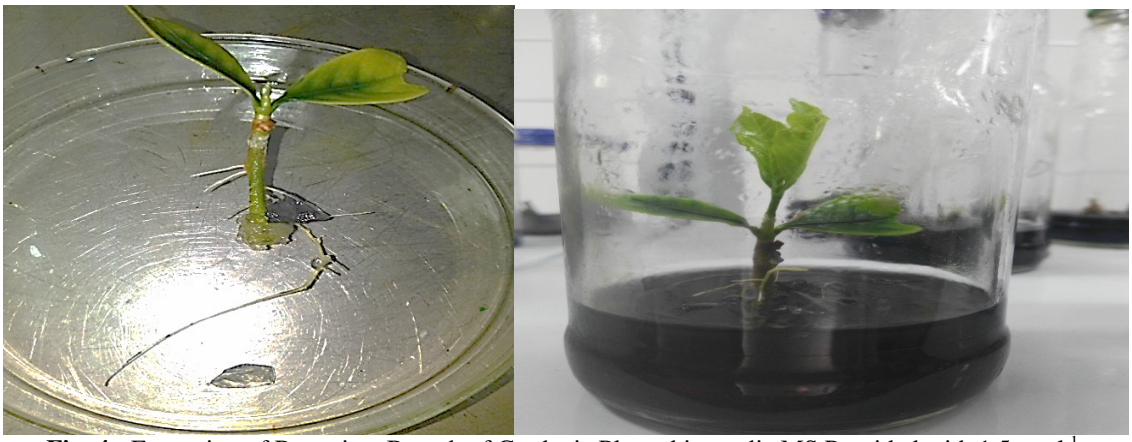

Fig. 4 : Formation of Roots in a Branch of Gardenia Planted in media MS Provided with 1.5 mg.l^{-1}.

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