Effect of growth regulators on growth and callus induction for Two Carnation (*Dianthus caryophyllus* L.) Cultivars in vitro.

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Abstract

This study was conducted in the Plant Tissue Culture Laboratory, Department of Horticulture and Landscape Gardening Engineering, College of Agriculture, University of Diyala, from 1 April 2025 to 15 January 2026 to evaluate the effects of BA and 2,4-D on shoot multiplication and callus induction in two carnation cultivars, the red cultivar 'Aveiro' and the pink cultivar 'Celebration'. BA was applied at 0, 1, 2, and 3 mg L^{-1} for shoot multiplication, while 2,4-D at 0, 1, and 2 mg L^{-1} was combined with BA at 0, 0.5, and 1 mg L^{-1} for callus induction. The results showed that 'Celebration' was superior in shoot and leaf numbers, whereas 'Aveiro' produced longer shoots. A BA concentration of 2 mg L^{-1} produced the highest number of shoots (8.444 shoots $plant^{-1}$), while 1 mg L^{-1} resulted in the longest shoots and the highest number of leaves (7.764 cm and 47.027 leaves $plant^{-1}$, respectively). For callus induction, the combination of 2 mg L^{-1} 2,4-D with either 1 or 0.5 mg L^{-1} BA achieved the highest callus induction percentage (100%).

Keywords: Carnation plant, Plant growth regulators, Callus, Ex vitro.

Introduction

Carnation (*Dianthus caryophyllus* L.) is one of the important species belonging to the family Caryophyllaceae. It is a perennial herbaceous plant that is commonly cultivated as an annual or semi-annual crop for commercial production purposes, particularly for producing its distinctive cut flowers used in floral arrangements. It is believed that the origin of carnation dates back to the Mediterranean Basin, where its natural distribution occurs in some regions of southern Europe, including France and Greece [1]. The genus *Dianthus* comprises approximately 300 plant species that vary in their growth habits, including perennial and annual herbaceous species, in addition to some species that grow as small shrubs. Carnation plants are characterized by a height ranging from 50–60 cm, opposite leaves with a thick texture and waxy surface, having a linear shape and a bluish-green color. Modern carnation cultivars are distinguished by continuous flowering, extended flower production periods, upright growth habit, and desirable morphological characteristics, making them among the most important commercial ornamental plants used for cut flower production [2]. Carnation is considered one of the important commercial ornamental crops. It is a dicotyledonous plant that grows successfully under temperate climatic conditions and is characterized by a wide

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range of flower colors, making it one of most demanded cut flowers in global markets. Developing cultivars with desirable floral traits and high productivity is one of the main objectives of crop improvement programs [3]. Vegetative plant parts are among the most reliable and successful explant sources for propagation using tissue culture techniques due to their genetic similarity to the mother plants [4]. Tissue culture has become one of advanced biotechnological techniques that contributes to the propagation of difficult-to-propagate plants and production of valuable pharmaceutical compounds. It also plays an important role in plant breeding programs by enabling the production of pure lines within short periods, the generation of haploid plants, the production of virus-free plants, and the development of new hybrids through the fusion of protoplasts isolated from genetically different plants [5].

Materials and Methods

The experiments were conducted in tissue culture laboratory of Department of Horticulture and Landscape Gardening Engineering, College of Agriculture, University of Diyala, during 1/4/2025 to 15/1/2026. The study included two carnation three months old. The culture medium was prepared using a ready-made medium at 4.9 g L^{-1} according to manufacturer's recommendations (HIMEDIA), supplemented with sucrose at 30 g L^{-1} and agar at 7 g L^{-1}. The culture medium and sucrose were added to 600 mL of deionized distilled water, and the components were thoroughly mixed. The pH of the medium was adjusted to 5.7 using sodium hydroxide (NaOH) solution. The volume was then completed to 1000 mL, and agar was added. The medium was heated until boiling to ensure complete dissolution of the agar, then dispensed into tissue culture vessels with a capacity of 350 mL and dimensions of 14 × 7 cm. The vessels were equipped with heat-resistant lids and a transparent window that allowed light penetration. After preparing the growth medium free of plant growth regulators and dispensing it into test tube, the media were sterilized using a steam pressure sterilizer (Autoclave) at a temperature of 121°C and a pressure of 1.04 kg cm^{-2} for 15 minutes to establish a sterile initiation culture as a preliminary step for subsequent experiments. All subsequent procedures were performed under aseptic conditions inside a laminar airflow cabinet (Laminar Air Flow Cabinet). The instruments used, including scalpels and forceps, were sterilized after being wrapped in heat-resistant aluminum foil using at 121°C and 1.04 kg cm^{-2} pressure for 30 minutes. Ethyl alcohol at a concentration of 99% was also used for sterilizing tools during manipulation inside cabinet, along with exposure to ultraviolet radiation to maintain aseptic conditions and minimize the possibility of contamination.

Tissue Culture Experiments

1. Sterilization Experiment and Preparation of Plant Explants

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Vegetative shoots (10–15 cm) were collected from red cultivar Aveiro and pink cultivar Celebration and transferred to the laboratory. The shoots were sprayed with 70% ethanol, cut into 3 cm segments, washed under water for 30 min, and cleaned with liquid soap after leaf removal. Surface sterilization was performed inside a laminar airflow cabinet using 10% (v/v) commercial bleach containing 6% active sodium hypochlorite supplemented with Tween 20. Nodal segments (≈1 cm) were exposed to sterilizing solution for 10, 15, or 20 min, then rinsed three times with sterile distilled water for 5 min each. Damaged tissues were removed, In MS media. Cultures were incubated at 25 ± 2°C under a 16 h light/8 h dark photoperiod. contamination and explant response percentages were recorded according to [6].

2. Effect of Different Concentrations of Benzyl Adenine (BA) on Shoot Multiplication of Two Carnation Cultivars Grown Ex Vitro

A two-factor factorial experiment with ten replicates was conducted to evaluate the effect of BA concentrations (0, 1, 2, and 3 mg L^{-1}) on shoot multiplication of two carnation cultivars. A single nodal explant was cultured in each test tube containing MS medium supplemented with the respective BA concentration. Cultures were maintained at 25 ± 2°C under a 16 h light/8 h dark photoperiod with a light intensity of 1000 lux. After four weeks, shoot multiplication was evaluated by recording number of shoots (shoot $plant^{-1}$), shoot length (cm), and number of leaves (leaf $plant^{-1}$)

3. Effect of 2,4-D in Combination with BA on Callus Induction Percentage of Two Carnation Cultivars Grown In Vitro

A factorial experiment with ten replicates was conducted to evaluate the effects of two carnation cultivars (pink and red), 2,4-D concentrations (0, 1, and 2 mg L^{-1}), and BA concentrations (0, 0.5, and 1 mg L^{-1}) on callus induction. A 1 cm^2 leaf explant was cultured on MS media supplement with combination of plant growth regulatoer incubated for four weeks at 25 ± 2°C under a 16 h light/8 h dark photoperiod with a light intensity of 1000 lux. Callus induction percentage was calculated as the number of explants producing callus relative to the total number of cultured explants × 100, and callus size was also recorded

Experimental Design

All experiments were conducted according to a Completely Randomized Design (CRD), with 10 replicates assigned for each treatment in the laboratory experiments. The data were statistically analyzed using the statistical software package SAS. Mean comparisons were performed using

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Duncan's Multiple Range Test (DMRT) at a significance level of 0.05.

Results and Discussion

1. Sterilization Experiment

The results presented in table (1) indicate the presence of significant differences in sterilization percentages of two carnation cultivars when different sterilization durations (10, 15, and 20 minutes) were applied. The 20-minute duration resulted in the highest sterilization percentage, reaching 95%, whereas the 10-minute duration recorded the lowest sterilization percentage (65%). Regarding the effect of cultivars on sterilization efficiency, the results of the same table revealed significant differences between the cultivars. The pink cultivar exhibited the highest sterilization percentage (83.3%), while the red cultivar recorded a sterilization percentage of 76.6%.Concerning the interaction between sterilization duration and cultivars, significant differences were observed. The pink cultivar treated for 20 minutes showed the highest sterilization percentage, reaching 100%, whereas the red cultivar treated for 10 minutes recorded the lowest sterilization percentage (60%).

Table (1) Effect of combination between cultivars and sterilization duration on the sterilization percentage after 7 days

Average duration	Cultivar		
	Red	Pink	Durations
65 B	60 c	70 bc	10
80 AB	80 b	80 b	15
95 A	90 ab	100 a	20
	76.6 B	83.3 A	Cultivar an

Means followed by the same letter for the two studied factors and their interaction are not significantly different from each other according to Duncan's Multiple Range Test at ($P \leq 0.05$).

2. Shoot Multiplication Experiment

2-1. Effect of Different BA Concentrations on Shoot Multiplication of Two Carnation Cultivars After Four Weeks of In vitro Culture

2-1-1. Number of Shoots (shoot plant^{-1})

Results presented in table (2) revealed significant differences between the carnation cultivars when different concentrations of BA were applied. The pink cultivar recorded the highest number of shoots, reaching 8.166 shoots plant^{-1}, whereas the red cultivar exhibited the lowest number of shoots,

ISSN 2072-3857

with 6.367 shoots plant^{-1}. Regarding the effect of BA application, the same table showed significant differences among the tested BA concentrations. The concentrations of 1 and 2 mg L^{-1} BA resulted in the highest number of shoots, reaching 7.736 and 8.444 shoots plant^{-1}, respectively. However, the number of shoots decreased at the concentration of 3 mg L^{-1} BA, which recorded 6.000 shoots plant^{-1}.Concerning the interaction between cultivars and BA concentrations, significant differences were observed. The pink cultivar treated with 1 and 2 mg L^{-1} BA produced the highest number of shoots, reaching 8.222 and 11.111 shoots plant^{-1}, respectively. In contrast, the red cultivar treated with 2 mg L^{-1} BA recorded the lowest number of shoots (5.777 shoots plant^{-1}).

Table (2). Effect of BA concentrations on the number of shoots of two Carnation cultivars grown in vitro

Average duration	BA mg L^{-1}				Duration
	3	2	1	0	
8.166 A	5.777 b	11.111 a	8.222 a	7.555 b	Celebration
6.367 B	6.222 b	5.777 b	7.250 b	6.222 b	Aveiro
	6.000 B	8.444 A	7.736 A	6.888 AB	mean BA

Means followed by the same letter for the two studied factors and their interaction are not significantly different from each other according to Duncan's Multiple Range Test at ($P \leq 0.05$).

2-1-2. Shoot Length (cm)

The results presented in Table (3) showed significant differences between the carnation cultivars when different concentrations of BA were applied. The red cultivar recorded the highest shoot length, reaching 7.493 cm, whereas the pink cultivar recorded the lowest shoot length, with 6.391 cm.

Regarding the effect of BA, the same table revealed significant differences among the tested BA concentrations. The concentration of 1 mg L^{-1} BA Showed in the highest shoot length, Reaching 7.764 cm. Meanwhile, the control treatment recorded the lowest shoot length (6.583 cm) and did not differ significantly from the other concentrations of 2 and 3 mg L^{-1} BA, which recorded 6.638 and 6.783 cm, respectively. Concerning the interaction between cultivars and BA concentrations, significant differences were observed. The red cultivar treated with 3 mg L^{-1} BA exhibited the highest shoot length, reaching 8.300 cm, whereas the pink cultivar treated with 3 mg L^{-1} BA recorded the lowest shoot length (5.2667 cm).

ISSN 2072-3857

Table (3) Effect of BA concentration and cultivars combination on shoot length for two Carnation cultivars in vitro.

Average duration	BA mg L^{-1}				Duration
	3.0	2.0	1.0	0.0	
6.3917 B	5.266 d	6.433 bcd	8.166 a	5.700 cd	Celebration
7.493 A	8.300 a	6.844 abc	7.362 ab	7.466 ab	Aveiro
	6.783 B	6.638 B	7.764 A	6.583 B	mean BA

Means followed by the same letter for the two studied factors and their interaction are not significantly different from each other according to Duncan's Multiple Range Test at ($P \leq 0.05$).

2-1-3. Number of Leaves (leaf plant^{-1})

The results in table (4) the pink cultivar recorded the highest number of leaves, reaching 48.416 leaves plant^{-1}, whereas the red cultivar recorded the lowest number of leaves, with 36.125 leaves plant^{-1}. Regarding the effect of BA concentrations, significant differences were observed among the tested concentrations. The highest number of leaves was obtained with the addition of 1 mg L^{-1} BA, reaching 47.027 leaves plant^{-1}. This treatment did not differ significantly from the control treatment and 2 mg L^{-1} BA, which recorded 44.000 and 43.166 leaves plant^{-1}, respectively. The concentration of 3 mg L^{-1} BA resulted in the lowest number of leaves, reaching 34.888 leaves plant^{-1}. The interaction between cultivar and BA concentration significantly affected leaf number, with the pink cultivar treated with 2 mg L^{-1} BA recording the highest value (54.333 leaves plant^{-1}), whereas the red cultivar treated with 3 mg L^{-1} BA recorded the lowest (26.777 leaves plant^{-1}).

Table (4) Effect of BA concentrations on the number of leaves of two Carnation cultivars grown in vitro

Average Duration	BA mg L^{-1}				Duration
	3	2	1	0	
48.416 A	43.000 b	54.333 a	49.555 ab	46.777 ab	Celebration
36.125 B	26.777 d	32.000 cd	44.500 ab	41.222 bc	Aveiro
	34.888 B	43.166 A	47.027 A	44.000 A	mean BA

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Means followed by the same letter for the two studied factors and their interaction are not significantly different from each other according to Duncan's Multiple Range Test at ($P \leq 0.05$).

3. Callus Induction Percentage

3-1. Effect of the Interaction Between 2,4-D and BA on Callus Induction Percentage of Two Carnation Cultivars

The results presented in table (5) showed that there were no significant differences between carnation cultivars in percentage of callus induction. The addition of different BA concentrations to culture medium had a significant effect on the percentage of callus induction. The concentration of 1 mg L^{-1} BA resulted in highest callus induction percentage, reaching 60%, compared with control treatment, which failed to induce callus formation. The addition of 2,4-D to culture medium at 2 mg L^{-1} resulted in highest callus induction percentage, reaching 100%, compared with control treatment, which did not result in callus formation. Regarding interaction between 2,4-D and BA concentrations, significant differences were observed among treatments in callus induction percentage. The treatment of 2 mg L^{-1} 2,4-D combined with 1 mg L^{-1} BA, and 2 mg L^{-1} 2,4-D combined with 0.5 mg L^{-1} BA, resulted in highest callus induction percentage (100% for each treatment) compared with control treatment, which failed to stimulate callus initiation.

Table (5). Effect of the interaction between 2,4-D and BA on callus induction percentage (%) of two Carnation cultivars grown ex vitro.

Mean of 2,4-nd BA	Aveiro	Celebration	BA	2,4-D
0 C	0 d	0 d	0.0	0.0
0 C	0 d	0 d	0.5	
60 C	60 c	60 c	1.0	
65 B	70 bc	60 c	0.0	1.0
90 A	100 a	80 abc	0.5	
90 A	90 ab	90 ab	1.0	
100 A	100 a	100 a	0.0	2.0
100	100	100	0.5	

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A	a	a		
100 A	100 a	100 a	1.0	
	65.55 A	65.55 A	Cultivars mean	

Means followed by the same letter for the two studied factors and their interaction are not significantly different from each other according to Duncan's Multiple Range Test at ($P \leq 0.05$).

The results showed that sterilization durations used in this study were within the commonly applied and accepted range in tissue culture techniques for carnation and ornamental plants in general. Several studies have indicated that the use of sodium hypochlorite at appropriate concentrations and exposure durations provides effective sterilization without affecting cell viability or their ability to divide and grow **[7]**.

The significant differences observed between the red and pink cultivars can be attributed to the genetic variation between them, which was reflected in their physiological responses under tissue culture conditions. Despite the standardization of the culture medium, type and concentration of growth regulators, and environmental conditions, including light intensity and temperature, each cultivar exhibited a distinct growth response, indicating the important role of genetic constitution in determining response efficiency, growth rate, and regeneration capacity **[8]**. These results agree with **[9]**who reported that sterilization of carnation explants using 10% sodium hypochlorite (NaOCl) at different exposure durations resulted in contamination-free explants, with some plants showing complete elimination of contamination after 4 minutes of sterilization. The results presented in tables (2), (3), and (4) indicate that the significant differences between the red and pink carnation cultivars were mainly attributed to their genetic differences. Genetic factors influence vegetative growth efficiency, cell division rate, and shoot formation, which consequently affect the number of leaves produced. These differences may also be associated with variation in cultivar responses to plant hormones, environmental conditions, and nutritional factors **[10]**. The superiority of some BA treatments in carnation shoot multiplication can be attributed to the stimulatory effect of cytokinin through enhancing cell division. Cytokinins also reduce apical dominance and promote nutrient translocation toward lateral buds, consequently stimulating their growth and development **[11]**. These results are consistent with **[12]**, who found that the optimum BA

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concentration for carnation shoot multiplication was 1 mg L^{-1}. Regarding shoot length, lower BA concentrations resulted in a slight increase in shoot elongation compared with higher concentrations.

The reduction in shoot elongation at higher BA concentrations may be attributed to the ability of cytokinins to promote cell division and lateral bud development at the expense of internode elongation, in addition to disrupting the hormonal balance with auxins [13]. Conversely, lower BA concentrations enhanced leaf production by stimulating cell division, promoting leaf initiation, and delaying leaf senescence. The variation among cultivars reflects genetic differences in their responsiveness to cytokinin. These results are consistent with those of [14], who identified 1.5 mg L^{-1} BA as the optimum concentration for shoot growth.
The results of Tables (4–5) and (4–6) indicate that efficient callus induction depends on a balanced interaction between auxin and cytokinin, whereas increasing one regulator at the expense of the other adversely affects callus development. Auxins initiate cell dedifferentiation and stimulate RNA synthesis required for cell division, while cytokinins enhance protein synthesis and cell proliferation [15,16]. The absence of callus formation without 2,4-D confirms the essential role of auxin during the initial stage of callus induction, whereas BA alone was insufficient to induce callus. The highest callus induction was achieved by combining 2,4-D with BA due to their synergistic action, where auxin initiates callus formation and cytokinin enhances subsequent cell division and biomass accumulation [17]. The superiority of higher 2,4-D concentrations suggests that the tissues remained within the optimal physiological range without inhibitory effects. These findings agree with [18], who reported that MS medium supplemented with 1 mg L^{-1} BA and 2 mg L^{-1} 2,4-D produced the highest callus induction.

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