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Callus Induction of *Dianthus caryophllus* L. and Economic Importance

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Abstract

The medicinal knowledge of herbal plants plays a crucial role in the discovery of new drugs. Among the Caryophyllaceae family, *Dianthus caryophyllus* (carnation) is an ornamental plant widely valued for its colorful flowers and pleasant fragrance. Beyond its aesthetic appeal, the plant possesses pharmacological and aromatic properties that make it useful in traditional folk medicine. The present study aimed to investigate the effect of different plant growth regulators on callus induction and fresh weight production in *D. caryophyllus* using *in vitro* plant tissue culture techniques. Seeds of *D. caryophyllus* were surface sterilized using various concentrations of sodium hypochlorite (NaOCl) for two exposure times (5 and 10 minutes). Explant (leaf, and node) were cultured on Murashige and Skoog (MS) medium supplemented with 2 types of auxins ,α-Naphthaleneacetic (NAA), 2,4-Dichlorophenoxy Acetic Acid (2,4-D) and cytokinins 6-benzyladenine (BA), Kinetin(KIN) at different concentrations. Callus induction was evaluated after 4 weeks of culture. Seeds were best sterilized by soaking in 3% NaOCl for 5 minutes. Leaf explants produced the highest (100%) callus induction rate on MS medium supplemented with 2 mg/L 2,4-D and 1.5 mg/L KIN, as well as node explants. The highest fresh weight of callus recovered from nodes was 0.92 g, while leaves had a fresh weight of 0.86 g. no callus induction was observed from internode explants. Growth regulators significantly influenced callus induction and fresh weight production in *D. caryophyllus*. The combination of 2 mg/L 2,4-D and 1.5 mg/L KIN have reported results for callus formation, particularly from node and leaf explants, providing a useful model for further experimental studies.

Keywords: plant tissue culture, *Dianthus caryophllus*, carnation,2,4-D, KIN, NAA, BA.

1. Introduction

Plant tissue culture has been used since 1902 to produce healthy, disease-free plants with the highest quality at a faster rate of production throughout the year, regardless of the season[1,2]. Every plant has a unique shape and set of dietary needs. Furthermore, the tissues in each section of the plant have different nutritional needs[3]. Economically beneficial plants are recently regarded as genetically enhanced and multiplied basis to the tissue culture process[4,5]. Tissue culture has been used to study various aspects of plant physiology, growth, increase secondary metabolism[6,7], reproduction[8], and nutritional needs under carefully controlled conditions. The growth of many plants in the absence of seeds or necessary pollinators for the formation of seeds[10]. Carnation, scientifically known as *Dianthus caryophyllus* belongs to the Caryophyllaceae family, refers to as a carnation and was developed approximately 2,000 years ago. It is not widely distributed over the world but is thought to have originated in the Mediterranean. The carnation is one of the most commercially important flowers and is regarded equally important as Roses. *D. caryophyllus* is a perennial herbaceous plant, reaching a height of up to 80 cm. The leaves are soft, grey to blue-green and up to 15 cm in length[11]. The base of *D.*

caryophyllus is covered in woody stems, with herbaceous branches emerging from it. It has medicinal properties[12]. They have largely become used in the treatment of tooth and stomachaches, as well as in the management of ailments like vermifugal, cardiac, and pulmonary disorders. Conventionally, in China, Japan, Korea, and other countries, carnations are used to treat wounds, gastrointestinal issues, and other illnesses [13,14].

D. caryophyllus contains many secondary metabolites that help treat various diseases in pharmaceuticals, including antimicrobial, antioxidant, antifungal, anti-viral, insecticide, anti-cancer, repellent, rheumatoid, and analgesic effects. Alkaloids, triterpenes, coumarins, phenolic acids, anthocyanins, essential oil, pelargonidin, isosalipurposide, volatile oil, and other soluble and/or insoluble compounds are present; for the studies, [15] and [16] employed nodal and leaf explants, respectively, as plant tissue sources to be determind, using complex combinations of BA (benzyladenine) and 2,4-Dichlorophenoxy Acetic Acid (2,4-D), the most appropriate seedling callus induction of *Dianthus caryophyllus* or carnation, while the study [17] examined the use of petal explants on MS media, using a combination of 1.5 mg\ L 2.4-D and 0.5 mg\ L NAA, achieving 100% callus induction. Explant types and PGRs have been proven to be crucial in inducing callus in *D.caryophyllus*. The objective of the present study is to examine how plant hormones and various leaf, node, and stem explants can induce callus in *D.caryophyllus*.

2. Materials and Methods

The experiments in this study were conducted in the Plant Tissue Culture Laboratory of the Biology Department at the College of Science for Women, University of Baghdad .

2.1. Explant collection and sterilization

2.1.1. Plant material

Seeds of *Dianthus caryophyllus* were procured fully labeled ,displaying the scientific name of the plant species and its image sourced from the local market in Baghdad. The seeds were then washed with tap water for 30-60 minutes and transferred to the air flow room cabinet. The seeds were then surface sterilized with sodium hypochlorite 6% with distilled Water.

DW 3%, 2%, and 1.5% concentrations for 5 or 10 minutes, then rinsed with sterilized DW for 5 minutes three times, then cultured on MS medium with 10 replicates. After 14 days of cultured, the germination percentage, the number of days required for germination, and the length of the seedlings were calculated for ten replicates[18].

2.1.2. Culture media

Murashige and Skoog medium (MS) (1962)[18] was used to induce plantlets of *Dianthus caryopyllus* L. The pH was adjusted from 5.5 to 5.8 and then autoclave at 15 psi and 121°C. For callus induction the combination of (BA: NAA) (2,4D: KIN) concentration 0,1,2,3 and 0 0.5,1, 1.5) mg/L was used and incubated for 3days starting of culturing.

Seeds were sterilized then cultured in free MS medium in vials under sterile conditions in a Laminar air cabinet. next, they were incubated at 25 ± 2 °C in the growth chamber in a 16/8 photoperiod with a light intensity of 3000 lux. After three weeks, seed germination resulted in the formation of plantlets.Seed planting for germination had ten replicates.

The leaf, node, and internode segments of the plantlets were removed and grown on MS media using a combination of PGRs. They were placed in an incubator with a 16:8 photoperiod for a month and transplanted as ten replicates.

By using a sensitive balance, the fresh weight of the produced callus was measured, which allowed for the determination of the percentage of callus induction.

The percentage of seedling survival without contamination were calculated according to the following **Equations (1, 2)**:

$$\text{Percentage of survival} = \frac{\text{Number of survival plant}}{\text{Total number of cultured explants}} \times 100 \quad \textbf{(1)}$$

$$\text{Germination index} = \frac{\text{Number of seeds germinated on day}}{\text{Number of days from the start of the experiment to day}} \quad \textbf{(2)}$$

2.2. Statistical Analysis

Package for the Social Sciences (SPSS version 19) was used to detect the effect of difference factors in study parameters. Least significant difference-LSD was used to significant compare between means in The present study Analysis of Variation-ANOVA[19].

3. Results

3.1. Plant material sterilization

The results of the sterilization experiment, as reported by **Tables 1-3 and Figure 1**, have shown that the sterilization period had an impact on the germination rate, seedling length, and the number of days required for germination. It was observed the seeds sterilized for 5 minutes recorded the highest germination rate compared to seeds sterilized for 10 minutes. While shorter exposure to the sterilizing agent was Non significant to remove contaminants without directly harming the seed tissue's vitality, longer exposure was required. (10 minutes) resulted in a relatively lower germination rate. 5 minute exposure was most effective because it eliminated surface pathogens without harming seed viability, while lower concentrations were insufficient to prevent contamination. This is attributed to the fact that prolonged exposure to the sterilizing agent may produce toxic effects or inhibit some biological processes within the seeds.

Table 1. The effect of NaOCl on Seed germination rate (%)

Explant	**Time (min.)**	**NaOCl 1:1**	**NaOCl 1:2**	**NaOCl 1:3**	**Mean**
Seed	5	100	90	90	93.33
	10	90	80	80	83.33
Mean		95.00	85.00	85.00	--
L. S. D.	NaOCl: 7.02 * , Time: 6.61 * , NaOCl x Time: 11.85 * .				
* (P≤0.05).					

Table 2. The effect of NaOCl on the Number of days required for germination

Explant	Time (min.)	NaOCl 1:1	NaOCl 1:2	NaOCl 1:3	Mean
Seed	5	2.9	3.5	3.5	3.3
	10	1.9	1.0	1.0	1.3
Mean		2.40	2.25	2.25	--
L. S. D.	NaOCl: 0.281 NS , Time: 0.230 * , NaOCl x Time: 0.504 * .				
* (P≤0.05).					

Table 3 The effect of NaOCl on Seed germination lengths(mm)

Explant	**Time (min.)**	**NaOCl 1:1**	**NaOCl 1:2**	**NaOCl 1:3**	**Mean**
Seed	5	6.0	5.6	5.5	5.70
	10	5.6	5.4	5.5	5.50
Mean		5.80	5.50	5.50	--
L. S. D.	NaOCl: 0.437 NS , Time: 0.311 NS , NaOCl x Time: 0.697 NS .				
* (P≤0.05).					

Figure 1. Seedling of *Dianthus caryophyllus* grown on MS hormone-free medium after 14 days.

3.2. Callus induction

3.2.1 The effect of NAA, BA, and the type of explant on the percentage and fresh weight of callus induction

The results reported by in **Tables 4-7 and Figure 2**, have indicated that both tissue type and NAA/BA combination significantly increased the callus induction percentage, as well as fresh weight. Nodes once again produced the highest induction percentage at (1 NAA mg/L), averaging 57%. Nodes also produced the highest fresh weight (0.92g) at the combination of (1.0 NAA, 1.0 BA mg/L) compared to all other treatments. The Leaves represented the lowest response overall; however, the combination of (1.0 NAA+1.0 BA mg/L) presented a percentage response of 70% and a fresh weight of (0.80 g). The interaction effect (tissue×combination) was statistically significant, indicating that the most effective combination of hormones varies According to the tissue source.

Table 4. The effect of BA and NAA on Percentage of Callus Induction from node (%)

BA (mg/L)	**NAA (mg/L)**				**Mean**
	0	**1**	**2**	**3**	
0	10.00	57.00	10.00	0.00	19.25
1	25.00	40.00	30.00	25.00	30.0
2	15.00	20.00	50.00	0.00	21.25
3	12.00	20.00	37.00	0.00	17.25
Mean	15.50	34.25	31.75	6.25	--
L. S. D.	BA: 7.18 * , NAA: 7.18 * , BA x NAA: 12.67 * .				
* (P≤0.05).					

Table 5. The effect of BA and NAA on the Fresh weight of callus from the node

BA (mg/L)	**NAA (mg/L)**				**Mean**
	0	**1**	**2**	**3**	
0	0.52	0.55	0.58	0.00	0.412
1	0.79	0.92	0.90	0.88	0.872
2	0.40	0.50	0.53	0.00	0.357
3	0.53	0.52	0.28	0.00	0.332
Mean	0.560	0.622	0.572	0.220	--
L. S. D.	BA: 0.269 * , NAA: 0.269 * , BA x NAA: 0.480 * .				
* (P≤0.05).					

Table 6. The effect of BA and NAA on Percentage of Callus from leaves (%)

BA (mg/L)	NAA (mg/L)				Mean
	0	1	2	3	
0	0.00	35.00	0.00	0.00	8.75
1	0.00	70.00	40.00	0.00	27.50
2	0.00	0.00	0.00	0.00	0.00
3	0.00	0.00	0.00	0.00	0.00
Mean	0.00	26.25	10.00	0.00	--
L. S. D.	BA: 5.94 * , NAA: 5.94 * , BA x NAA: 9.53 * .				
* (P≤0.05).					

Table 7. The effect of BA and NAA on the Fresh weight of callus from leaves

BA (mg/L)	NAA (mg/L)				Mean
	0	1	2	3	
0	0.00	0.50	0.00	0.00	0.125
1	0.00	0.80	0.61	0.00	0.352
2	0.00	0.00	0.00	0.00	0.00
3	0.00	0.00	0.00	0.00	0.00
Mean	0.00	0.325	0.152	0.00	--
L. S. D.	BA: 0.184 * , NAA: 0.184 * , BA x NAA: 0.327 * .				
* (P≤0.05).					

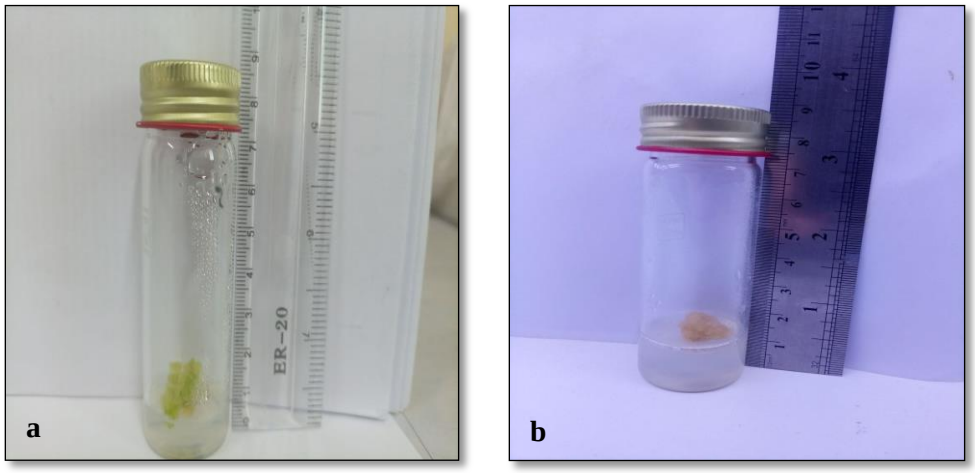

Figure 2. The amount of callus induction from different explants of seedlings with various concentrations of hormones (a) node (1 mg/L NAA and 1 mg/L BA), (b) leaf (1 mg/L NAA and 1 mg/L BA)

3.2.2 Callus induction percentage and fresh weight are negatively impacted by 2,4-D and kin concentration and explant type

The result in **Tables 8-11 and Figure 3** have shown the effect of 2,4-D and kin in the percentage and fresh weight of callus induction from nodes and leaf, the consequences reported by in this table display that in some amalgamation of kin and 2,4-D, the percentage rate of callus was 100%after one month, and fresh weight reached 0.86g at 2 mg\L 2,4-D,1.5 mg\L Kin from node, while leaf percentage callus was 100% and fresh weight 0.64g, under the same treatment. Increasing and altering the concentration of auxin increased the percentage of explants demonstrating callus induction but simultaneously reduced the fresh weights. No other combinations were significantly different. 2,4-D and NAA demonstrate callus induction on explants.

Table 8. The effect of KIN and 2, 4-D in Percentage of Callus induction from internode (%)

KIN (mg/L)	**2, 4-D (mg/L)**				**Mean**
	0	1	2	3	
0	10.00	50.00	20.00	30.00	27.50
0.5	25.00	30.00	30.00	25.00	27.50
1	40.00	25.00	30.00	100	48.75
1.5	50.00	50.00	100	10.00	52.50
Mean	31.25	38.75	45.00	41.25	--
L. S. D.	KIN: 6.72 * , 2, 4: D: 6.72 * , KIN x 2, 4-D: 11.45 ** .				
* (P≤0.05).					

Table 9. The effect of KIN and 2, 4-D on the Fresh weight of Callus induction from internode

KIN (mg/L)	2, 4-D (mg/L)				Mean
	0	1	2	3	
0	0.49	0.55	0.50	0.45	0.497
0.5	0.50	0.60	0.62	0.48	0.550
1	0.46	0.55	0.61	0.34	0.490
1.5	0.45	0.73	0.86	0.18	0.555
Mean	0.475	0.607	0.647	0.362	--
L. S. D.	KIN: 0.207 NS , 2, 4- D: 0.207 * , KIN x 2, 4-D: 0.387 * .				
* (P≤0.05).					

Table 10. The effect of KIN and 2, 4-D on Percentage of Callus Induction from leaves (%)

KIN (mg/L)	**2, 4-D (mg/L)**				**Mean**
	0	1	2	3	
0	0.00	50.00	50.00	80.00	45.00
0.5	40.00	50.00	50.00	50.00	47.50
1	12.00	60.00	57.00	45.00	43.50
1.5	10.00	80.00	100	40.00	57.50
Mean	15.50	60.00	64.25	53.75	--
L. S. D.	KIN: 8.19 * , 2, 4- D: 8.19 * , KIN x 2, 4-D: 14.31 * .				
* (P≤0.05).					

Table 11. The effect of KIN and 2, 4-D on the Fresh weight of Callus induction from leaves

KIN (mg/L)	**2, 4-D (mg/L)**				**Mean**
	0	1	2	3	
0	0.00	0.43	0.47	0.42	0.330
0.5	0.36	0.42	0.48	0.41	0.417
1	0.33	0.44	0.47	0.43	0.417
1.5	0.50	0.56	0.64	0.46	0.540
Mean	0.297	0.462	0.515	0.430	--
L. S. D.	KIN: 0.216 * , 2, 4- D: 0.216 * , KIN x 2, 4-D: 0.371 * .				
* (P≤0.05).					

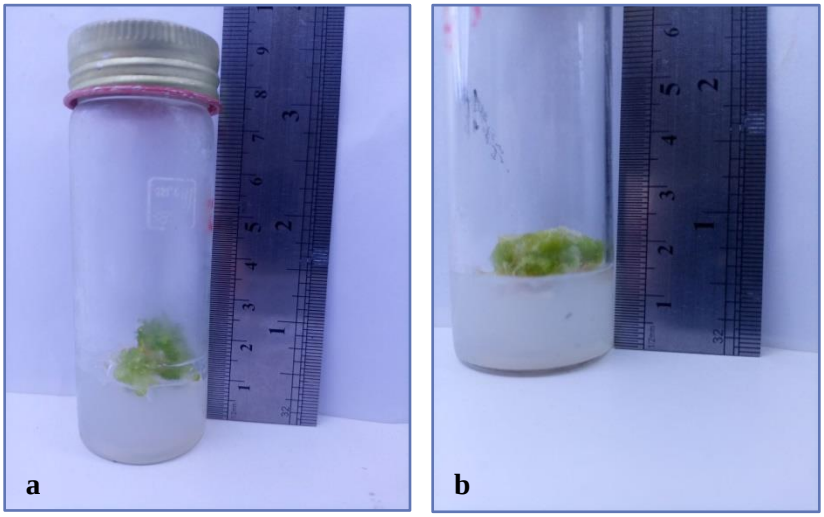

Figure 3. The amount of callus induction from different explants of seedlings with various concentrations of hormones (a) node (2 mg/L 2,4-D and 1.5 mg/L/LKIN) (b) leaf (2 mg/L 2,4-D and 1.5 mg/L KIN)

4. Discussion

The results shown by in Tables 1-4 can have reported that continuous exposure to the sterilizing agent may have toxic effects or may have disrupted some biological processes pertaining to the seeds. This is in agreement with the results of study[20]. In terms of the number of days until germination, there was a minimal number of days for seeds directed sterilized for 5 minutes, as they began to germinate at a higher rate, while seeds sterilized for 10 minutes took longer to germinate. In this regard when sterilized for a longer period of time, the biological activity is delayed in terms of initiating germination, which is consistent with the results of study[21]. In terms of the growth from the above treatments, the seeds that were sterilized for 5 minutes exhibited more length of the shoot compared to the treatments that were sterilized for 10 minutes, meaning that for a duration of time, the conditions were better for normal seedling growth. For the seeds that received a longer duration of sterilization there was a comparative delay in growth and development, which may be suggestive of the sterilizing agent affecting the activity of meristematic cells. This confirms with study[22].

Regarding the effect of NAA, BA different, and type the interaction effect (tissue × combination) was statistically significant reflecting the optimal hormonal pattern required differed depending upon tissue sources[23]. Nodes responded at times better than leaves; The recent study suspected nodes had meristematic areas with a greater density of cells than leaf tissue that were more capable of dividing and differentiating leaf tissue. When either NAA or BA concentrations increased independently, the callus response were decreased. This could be explained by having one of the two accumulating to high levels, having an inhibitory effect, and an ideal hormonal ratio. This increase in fresh weight of callus with (NAA 1.0 + BA 1.0) would respond adequately because this concentration increased cell division and accumulation of living matter, reflected in the size and weight of callus; these findings are consistent the studies [24, 25].

For the effect of 2,4-D and kin in various concentrations, the dominance of 2,4-D over NAA in inducing callus from explants may be due to the chemical constituents of the growth regulators and its their subsequent effect on the metabolic reactions in the explants, including their impact on the accessibility of nutrients[26]. These conclusions are similar to the outcome of [27, 28] where 2,4-D and kin are superior to IAA in callus induction. Moreover, the differences in responses to the explants, competing induced the callus, is mostly still due to physiological and anatomical differences of the explants regarding the degree of ripeness of thier cells. A possible change would be differences in the contented of plant hormones and nutrients in quantity and quality [29, 30]. The lower concentration of either auxin or cytokinin is likely due to the type of auxin, its concentration, or both inadequacies in inducing favorable conditions for cell renewal

(totipotency), especially since meristematic cells cease growing in the deficiency of any growth factor [31].

5. Conclusion

In light of the results obtained, we concluded that 2,4-D and KIN are the most efficacious in stimulating callus. The nodes are the most favorable tissue for inducing callus from the leaf. The optimum medium for the differentiation of callus into shoots is the MS nutrient medium supplemented with 2 mg/L 2,4-D and 1.5 mg/L kinetin mg/L.

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Conflict of Interest

The authors declare that they have no conflicts of interest.

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Ethical Clearance

This study involved only plant materials (*Dianthus caryophyllus*) and did not include any experiments on humans or animals. Therefore, ethical approval was not required.

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