





EFFECT OF PLANT GROWTH REGULATORS ON IN VITRO AND EX VITRO CONDITIONS ON PROPAGATION OF DIANTHUS CARYOPHYLLUS L

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Abstract: This study was carried out in the tissue culture Laboratory at University of Baghdad during 2017-2018 in order to evaluate the effect of plant growth regulators on the propagation of two cultivars of Dianthus (Sun charm White and Master) under Ex vitro conditions by cultivating the single nodes using the MS medium which involved with different concentrations of growth regulators. The interaction treatment 1.0 mg /L BA + 0.2 mg /L NAA gave the highest response percent of bloom reached 100% for the red cultivar, and the treatment 1.5 mg / L BA + 0.2 mg / L gave the highest response percent to the white cultivar bloom reached 90%. In the multiplication stage, the highest branches number was obtained at 4.5 branches by the application of 2.0 mg / L BA + 0.2 and 0.4 mg /L IAA for the red cultivar, while the white variety gave the highest branches number obtained at the treatment 2.0 mg /L BA + 0.1 mg /L IAA, which gave 4.8 branches. In the experiment of rooting under Ex Vitro conditions, the results revealed that the concentration of 2 mg / L IBA gave the highest rooting ratio, number, and length of roots reaching 50%, 1.40 and 1.23 cm respectively for the red cultivar, while the white cultivar was given, 70%, 1.60 and 1.20 cm respectively, and the plants were adapted under greenhouse conditions.

Keywords: Ex Vitro, Dianthus, MS, IBA.

1. Introduction

Dianthus caryophyllus belongs to Caryophyllaceae family, which is a perennial herbaceous plant. It was found as wild growth in the Mediterranean, with a branching height of 50-60 cm, with opposite thick waxy striped leaves, and flowers of many colors and aromatics, the diameter of flowers may reach more than 10 cm. It is one of the most important crops of cut flowers in the world due to the beauty of flowers; considered as the best in coordination for the available qualities and colors in all seasons of the year [1]. The technique of plant

tissue culture has been used in *Dianthus*'s propagation, but this technology is affected by several factors; including related to the nutrition medium and the physiological factor of the plant, and the success percent varies from season to another and according to the nature and age. The increment or decrement of the ideal limit affects the response of the plant organs while they are grown in tissue culture [2].

Several successful attempts were carried out to propagate *Dianthus* by planting different plant organs in tissue culture. [3] mentioned the use of single nodes of the *Dianthus* plant gave a successful plant. [4] recorded

the highest propagation of the of branches apical of the Dianthus plant when planted on the medium of MS which equipped with 3 mg / L BA reached 7 branches / plant organs and in percent of rooting peaked at 100% when planting on the medium of MS with a quarter of the strength of the concentration of minerals (0.1 mg / L IBA). [4] were recorded a number of branches of the white Dianthus plant Jeanne Dionis Blanco reached 30 branch/ plant of the nodes cultivation. The research aims to propagate the Dianthus plant and evaluate the response of the branches that resulting from the propagation of roots in Vitro and under the conditions Ex Vitro in the field.

2. Materials and Methods

The study was carried out at University of Baghdad during 2017-2018. The plant parts with the same nodes number were taken from the two cultivars, the white Dianthus (Sun charm white) and the red Dianthus (Master) 3cm from growing parents in the lath house that be-longs to the Plant Protection Research Center, Abu Gharib. The plant parts were transferred to the laboratory and kept under running water for 15 minutes, after washing with water and liquid soap to get rid of dust and suspended matter, then, all the leaves were re-moved and sterilized with a solution of Clorox 6% and a concentration of 3% (volume / volume) for 8 minutes with the addition of the diffuser, thereafter washed three times with sterile distilled water for 5 minutes each time after cutting the vegetable parts to a length of 1 cm and planted on the medium of MS equipped with concentrations of 0, 0.5, 1.0, 1.5 mg/L BA and NAA for developmental phase, one branch was planted in each tube and there were 10 replications per treatment and measurements were taken 6 weeks after planting.

The concentrations BA and IAA were applied for doubling stage with one branch per tube in total of ten tubes. The data was recorded after 6 weeks of cultivation. The cultivated parts were incubated in all stages of propagation at a temperature of 25 ± 2 and under light intensity of 1000 lux for 16 hours of light. The branches resulting from the doubling phase were planted in two rooting experiments; the first was in vitro, the green branches resulting from the doubling were cultivated on the medium of MS with full strength and equipped with concentrations of 0.0, 0.5, 1.0, 1.5 mg/L IBA [5]. The second was ex vitro by rooting the branches resulting from the cultivation experiment by putting the ends of the branches in IBA for 5 minutes and then planted directly in the culture medium consisting of sand and peat moss in a 1: 1 ratio taking into account their coverage until completion Rooting after 7-10 days have passed [5]. The plants were extracted from the pots and washed with water to get rid of the leftover nests sticking to their roots, after which the plants were immersed for 15 seconds with Beltanol (bacterial fungicide) at a concentration of 1 ml / L to prevent fungal infection.

The plants were planted in plastic pots with a diameter of 5 cm, filled with a medium consisting of peat moss and mixtures in a ratio of 1: 1 for 10 replicates per treatment. The pots were covered with transparent plastic covers and incubated in the growth room under the same environmental conditions that the cultivated samples incubated. After two weeks of planting, the covers were gradually opened, taking into account watering with MS solution, a quarter of the salt strength, and the covers were lifted permanently after 4 weeks of planting and transferred to the greenhouse. The experiments were carried out using Complete Randomized Design and the results were analyzed using the SAS [6].

3. Results and Discussion

Establishment stage: There were significant differences in the percent of nodes for the red cultivar with different concentrations (Table1). The application of NAA at 0.2 mg/L gave the highest average response of (67.5%), which is significantly differed from the rest of treatments; and lowest response was (27.5%) in 0.0 mg/L NAA. The application of BA at the concentration of 1.0 mg/L gave the highest response rate reached (70%) compared to the rest of the treatments. The interaction between BA and NAA, gave the highest response peaked at (100%) under treatment of 1.0 mg/L BA + 0.2 mg/L NAA compared to the 0.0 mg/L BA + 0.5 mg/L NAA which did not give any response. The application of NAA indicates a significant at concentration of 0.2 mg / L in the response rate, (62.5%) compared with the control (20.0%).

Table 1-A. Effect of NAA and BA in response percent of nodes / (Red Dianthus).

NAA	BA				Mean of NAA
	0	0.5	1.0	1.5	
0.0	0	30	40	40	27.5
0.1	0	50	70	60	45.5
0.2	30	60	100	80	67.5
0.4	10	40	70	50	42.5
Mean of BA	10.00	45.00	70.00	57.50	---

LSD value: NAA: 6.59 *, BA: 6.59 *, NAAxBA: 11.64 *, (P≤0.05).

The application of BA at 1.5 mg/ L gave significantly high response (62.50%), which is differed significantly as compared with the treatments. The concentration of 1.5 mg / l BA + 0.2 mg / l NAA gave the highest percent (90%) compared to the rest of the treatments and may be due to the availability of the ideal ratio between auxin and cytokine to occur the response.

Table 1-B. Effect of NAA and BA in response percent of nodes/ (White Dianthus).

NAA	BA				Mean of NAA
	0	0.5	1.0	1.5	
0	0	20	30	30	20.0
0.1	20	40	50	60	42.5
0.2	20	60	80	90	62.5
0.4	30	40	60	70	50.0
Mean of BA	17.0	40.00	55.00	62.50	---

LSD value: NAA: 7.02 *, BA: 7.02 *, NAAxBA: 12.97 *, (P≤0.05) *.

Growth parameters: Branches number: The application of IAA at 0.2 mg/L gave the highest average number of branches (2.70), which did not differ significantly from the 0.4 mg / L. BA at the concentration of 2.0 mg/L BA gave the highest average number of branches (3.62). The 2.0 mg / L BA + 0.1 mg / L IAA, significantly differed 2.0 mg / L BA + 0.4 mg / L IAA. The lowest branches number was in control which did not differ significantly 0.0BA + 0.1IAA, which gave the average number of branches 0.3 and 0.6 respectively.

Table 2. Effect of IAA and BA on number of branches.

IAA	BA				Mean of IAA
	0	1.0	2.0	3.0	
0	0.3	1.6	2.0	1.1	1.25
0.1	0.6	3.2	4.5	1.9	2.55
0.2	0.8	4.0	3.5	2.5	2.70
0.4	0.6	2.0	4.5	3.2	2.70
Mean of BA	0.575	2.70	3.62	2.18	---

LSD value: IAA: 0.663*, BA: 0.663*, IAAxBA: 1.047*, (P≤0.05)

The application of IAA and BA gave the highest branches at the concentration of 0.2 mg /L, (2.87), which did not differ significantly 0.1 mg /L in comparison IAA. The effect of BA at 2 mg / L gave the highest number of branches (3.67 compared) and 3.0 gave the lowest. The interaction 2.0 mg / L BA + 0.1 mg / L IAA gave the maximum branches (4.8) which did not differ significantly from 2.0 mg / L BA + 0.2 mg / L IAA. These may be due to BA which is cytokinins that have an important role in eliminating apical dominance and establishing sink areas in lateral shoots, thus increasing the number of branches on the cultivated part by increasing the concentration up to the optimum level.

Table 2-B. Effect of IAA and BA in mean of branches NO (White Dianthus).

IAA	BA				Mean of IAA
	0	1.0	2.0	3.0	
0	0.0	1.5	2.3	1.8	1.40
0.1	0.5	2.6	4.8	3.4	2.82
0.2	0.4	3.4	4.5	3.2	2.87
0.4	0.6	2.7	3.1	1.7	2.02
Mean of BA	0.37	2.55	3.67	2.52	---

LSD value: IAA: 0.633*, BA: 0.633*, IAAxBA: 1.147*, ($P \leq 0.05$)

The branches length: Results in table (3-A) revealed that there were no significant differences in the effect of IAA on the branches lengths of the red dianthus cultivar, as the control treatment gave the highest average length of the branches reached 2.19 cm. also the effect of BA, showed a significant difference, as the highest average length of branch at the concentration of 3.0 mg / l BA 2.54 cm, which did not differ significantly from the concentration of 1.0 mg / l BA, reached 2.51 cm.

Table 3-A. Effect of IAA and BA in mean of plant high/ (Red Dianthus).

IAA	BA				Mean of IAA
	0	1.0	2.0	3.0	
0	0.88	2.00	2.82	3.09	2.19
0.1	1.40	2.79	1.58	2.31	2.02
0.2	1.04	2.54	2.12	2.46	2.04
0.4	1.56	2.70	1.37	2.31	1.98
Mean of BA	1.22	2.51	1.97	2.54	---

LSD value: IAA: 0.308 NS, BA: 0.308*, IAAxBA: 0.589*, ($P \leq 0.05$).

As for the effects of BA and IAA concentrations on the branches lengths of the white dianthus cultivar, the results of Table (3-B) indicate the presence of significant differences. As for the effect of IAA, it is noticed that the highest average length of the branches was (2.52) at the concentration of 0.4 mg / l, which differed significantly from the rest of the treatments. As for the effect of BA the same table shows that the concentration at 1.0 mg / L BA gave the highest average length of the branches reached (2.53 cm). Perhaps the reason for this may be that the increase in the concentration of cytokines in the medium reduces the role of accumulated Auxin inside the branches responsible for the elongation of stem cells towards the longitudinal, causing a shortening the length of the branches. Also, the effect of the interaction at the treatment 3.0 mg / L BA + 0.0 mg / L IAA, gave the highest average length of the branches reached (2.92 cm), which did not differ significantly from the treatment 1.0 mg / L BA + 0.4 mg / L IAA which gave 2.88 cm.

Table 3-B Effect of IAA and BA in mean of high plant/ (White Dianthus).

IAA	BA				Mean of IAA
	0	1.0	2.0	3.0	
0	0.00	2.40	2.38	2.92	1.93
0.1	1.31	2.59	1.44	1.98	1.83
0.2	1.75	2.25	1.52	1.87	1.84
0.4	1.20	2.88	2.19	2.35	2.52
Mean of BA	1.06	2.53	1.88	2.28	---

LSD value: IAA: 0.575 *, BA: 0.575*, IAAxBA: 0.941*, ($P \leq 0.05$).

Rooting: Experiment with Rooting IN Vitro: Results in Table (4-A) revealed that there are significant differences for the effect of IBA on the number, length and percentage of rooting for the red dianthus cultivar. The same table shows that the concentration of 1.0 mg /L IBA gave the highest percentage of rooting and the highest root length of (70%) and (2.01) cm respectively. Compared to the control treatment, while the treatment 1.5 mg / L IBA gave the highest number of roots was (2.50).

Table 4-A Effect of IBA in No, High and percent of roots/ (Red Dianthus).

IBA	No of roots	High of roots	Percent of roots %
0.0	0.20	0.55	20
0.5	0.60	1.27	40
1.0	2.10	2.01	70
1.5	2.50	1.83	60
LSD value	0.508 *	0.566 *	8.271 *

* ($P \leq 0.05$).

On the other hand, results of the white dianthus cultivar, in Table (4-B) showed that there are significant

differences for the application of IBA in the number, length and percentage of rooting. The same table shows that the concentration of 1.0 mg / l IBA gave the highest percent of rooting and the highest root length of (80%) and (2.19 cm), respectively. Compared to the comparison treatment, while the treatment 1.5 mg / L IBA gave the highest number of roots (2.30).

Table 4-B. Effect of IBA in No, High and percent of roots (White Dianthus).

IBA	No of roots	High of roots	Percent of roots %
0.0	0.0	0.00	0
0.5	1.20	1.23	50
1.0	2.00	2.19	80
1.5	2.30	2.07	60
LSD value	0.458 *	0.586*	9.021 *

* (P≤0.05).

Experiment with Rooting EX Vitro: Results in Table (5-A) indicated that there are significant differences in the application of IBA on the number and length of roots and the percent of rooting for the red dianthus cultivar. The concentration of 2.0 mg / L gave the highest percentage of rooting reached 50% and the highest length and number of roots reached 1.23 cm and 1.40 respectively, which were higher compared to the rest of the interactions.

Table 5-A. Effect of IBA in No, High and percent of roots/ Red Dianthus (Exvitro exp.).

IBA	No of roots	High of roots	Percent of roots %
0.0	0.0	0.00	0
2.0	1.40	1.23	50
4.0	1.30	0.94	50
6.0	0.60	0.83	30
LSD value	0.433 *	0.562 *	7.649 *

* (P≤0.05).

Results of the white dianthus cultivar in Table (5-B) indicated that the IBA has a significant effect on the number and length of roots and the percent of rooting; the concentration 2.0 mg / L IBA gave the highest percent of rooting reached (70%) the highest number and length of root length of (1.20 cm) and (1.6) respectively. In general, Auxin has an important role in rooting. The primary root cells present in the base of the growing branches depend in their division on the concentrations of internal and external Auxins, which cause an increase in cell divisions or to transform the mature cells in the bases of the branches into Meristematic cells [7]. Perhaps the reason of applying the IBA to the rooting medium is that the IBA is characterized by slow decomposition due to the effect of the Auxin-analyzing enzymes, which is a desirable feature in keeping the Auxin longer in the tissues. Therefore, IBA is the best in rooting branches, and these results were in agreement with [8] that confirmed that IBA is the best in rooting branches of Gardenia plants.

Table 5-B. Effect of IBA in No, High and percent of roots/ White Dianthus (Exvitro exp.).

IBA	No of roots	High of roots	Percent of roots %
0.0	0.0	0.00	0
2.0	1.60	1.20	70
4.0	1.50	1.13	50
6.0	0.80	0.91	30
LSD value	0.417 *	0.463 *	8.702 *

* (P≤0.05).

Acclimatization: The percent of acclimatized plants reached 85% and 80% for the red and white cultivars respectively, and in the ExVitro experiment it reached 70% and 73% for red and white respectively, after 3 months of transportation. These ratios are good because the hardening stage is one of the critical stages compared to laboratory propagation. This result is in agreement with [9] The acclimatization rate for dianthus reached 76.92%.

4. Conclusion

In conclusion, the application of IBA at a concentration of 1 mg/L for the red and white cultivars obtained the highest rooting ratio and the highest root length reaching 70% and 2.01 cm respectively for red, the highest root number reaching 2.50 for red and 2.30 for white at a concentration of 1.5 mg/L. In an experiment involving rooting in vitro, a concentration of 2 mg/L IBA resulted in the highest rooting ratio, number, and length of roots, reaching 50%, 1.40, and 1.23 cm for the red cultivar and 70%, 1.60, and 1.20 cm for the white cultivar, respectively. The plants were then adapted in a greenhouse.

Supplementary Materials:

No Supplementary Materials.

Author Contributions:

I. R. Khairi and M. A. Salman; methodology, writing—original draft preparation, I. R. Khairi; writing—review and editing, M. A. Salman; paraphrasing. All authors have read and agreed to the published version of the manuscript.

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The study was conducted in accordance with the protocol authorized by the University of Anbar, Ethics Committee, Iraq in cooperation with The Islamic Republic of Iran, from a commercial farm, fertile eggs from Ross (308) strain broiler breeder hens were obtained.

Informed Consent Statement:

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The authors declare no conflict of interest.

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4. References

- [1] M. Higashiura, M. Douzono, Y. Uno, and M. Yamanaka, "Verification of the effects of end-of-day-cooling on floral differentiation and cut-flower characteristics in carnation (*Dianthus caryophyllus* L.)," *Horticulture Journal*, vol. 90, no. 3, 2021, doi: 10.2503/hortj.UTD-258.
- [2] M. M. Wei, J. M. Wang, I. Muhammad, and B. Hong, "Culture in vitro and plant regeneration from petals of flower color chimera in chrysanthemum," *Beijing Linye Daxue Xuebao/Journal of Beijing Forestry University*, vol. 36, no. 4, 2014, doi: 10.13332/j.cnki.jbfu.2014.04.020.
- [3] H. Salehi, "Can a general shoot proliferation and rooting medium be used for a number of carnation cultivars?," *Afr J Biotechnol*, vol. 5, no. 1, 2006.
- [4] J. Lu, X. Bao, M. Zhang, Y. Zhao, and Q. Alatan, "Effect of Growth Regulators on Rooting of Carnation (*Dianthus caryophyllus*) Tissue Culture Seedlings," *International Journal of Horticulture*, 2021, doi: 10.5376/ijh.2021.11.0001.

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- [5] P. Soundararajan, A. Manivannan, Y. S. Cho, and B. R. Jeong, "Exogenous supplementation of silicon improved the recovery of hyperhydric shoots in dianthus caryophyllus L. By stabilizing the physiology and protein expression," *Front Plant Sci*, vol. 8, 2017, doi: 10.3389/fpls.2017.00738.
 - [6] SAS, "Statistical Analysis System User's Guide," *SAS. Inst. Inc. Cary. N.C. USA.*, vol. 9.6, no. Version 9.1th ed, 2012.
 - [7] T. Sharma, M. Modgil, and M. Thakur, "Factors affecting induction and development of in vitro rooting in apple rootstocks," *Indian J Exp Biol*, vol. 45, no. 9, 2007.
 - [8] R. Farzinebrahimi, R. Mat Taha, K. Rashid, and J. Syafawati Yaacob, "The effect of various media and hormones via suspension culture on secondary metabolic activities of (Cape Jasmine) *gardenia jasminoides ellis*," *Scientific World Journal*, vol. 2014, 2014, doi: 10.1155/2014/407284.
 - [9] H. Yoshida *et al.*, "Alteration of methylation profiles in distinct cell lineages of the layers during vegetative propagation in carnations (*Dianthus caryophyllus*)," *Euphytica*, vol. 135, no. 2, 2004, doi: 10.1023/B:EUPH.0000014878.73338.ac.