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RESEARCH ARTICLE

Production of Lavender Plants on Vitro

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ABSTRACT

This experiment was carried out in the Plant Tissue Culture Laboratory of the Department of Plant Production Technologies / AL-Mosab Technical College / Al-Frat Al-Awsat Technical University from 9/1/2023 to 6/1/2024 to study the effect of some plant growth regulators: auxin (2,4-D) at concentrations (0.0 and 0.0. 5, 1, 1.5, and 2) mg L^{-1} and cytokine (BA) at concentrations (0.0, 1, 1.5, 2.5, and 2) mg L^{-1} and their interactions in the propagation of lavender plants outside the living body and their effect on the production of vegetative branches and the emergence of a vegetative and root group. And the experience of producing vegetative branches: (NAA) in concentrations of (0, 0.5, 1, 1.5, and 2) and(BA) in concentrations of (0, 1, 1.5, 2, and 2.5)mg L^{-1}.The known nutrient medium (M S) was used. This experiment was designed according to a completely randomized design (CRD) with five replicates. Then the means were compared using the least significant difference (LSD) below the level of Probability (0.05).

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INTRODUCTION

The lavender plant *(Lavandula angustifolia*) belongs to the mint family (*Lamiaceae*) and is one of the most widespread essential oil plants throughout the world. It is used in the food industries, perfumes, cosmetics, and in various branches of medicine (1). The original homeland of the lavender plant is in the Mediterranean region. But it is grown in many other countries in the world with a large number of beneficial properties. It is a well-known species since ancient times and because of the fragrances that lavender flowers give. It is called (levantica) by (2). There is a need to propagate delicate lavender to produce large quantities of ornamental plants. One of the most important problems that hinders the spread of lavender is that propagation through seeds is slow, and vegetative propagation is necessary to produce genetically homogeneous individuals, and the rootstock is ineffective. There are additional factors such as climate, availability of water, and its susceptibility to disease, as well as difficulty. Obtaining lavender, whether from seeds or any plant parts (3). Plant tissue culture has enabled us to obtain cultures of cells, tissues, sterile organs and even the entire plant on suitable nutrient media and under controlled conditions of heat, humidity and light (4) Growth regulators are among the most important factors in tissue culture added to the nutrient media to make the process of micro propagation of plants successful. Auxins and cytokines are among the most widely used growth regulators (5). Using micro propagation technology allows the production of large numbers of plants within a short period of time, and it is possible through these The technique is plant propagation, which is difficult to propagate using traditional methods. Ex vitro cultivation can be divided into four stages: the plant emergence stage, the multiplication stage, the rooting stage, and

the acclimatization stage (6) In order to ensure the medicinal and industrial importance of this plant, in addition to being an ornamental plant, the study aims to investigate the effect of growth regulators on callus induction (fresh weight and dry weight), the effect of growth regulators on the formation of the shoot, the effect of growth regulators on the formation of the root system, and the production of lavender ex vitro.

MATERIALS AND WORKING METHODS

This experiment was carried out in the plant tissue culture laboratory of the Department of Plant Production Technologies / AL-Mosab Technical College / Al-Frat Al-Awsat Technical University from 9/1/2023 to 6/1/2024 to study the effect of some plant growth regulators and the interaction between them on the propagation of lavender plants ex vitro. Auxin (NAA) in concentrations (0.0, 0.5, 1, 1.5, and 2) mg L^{-1}, and cytokine (BA) in concentrations (0.0, 1, 1.5, 2.5, and 2) mg L^{-1} and their interactions in the propagation of lavender plants ex vivo and their effect on the production of vegetative shoots and group emergence. Vegetative and rooted, I took parts of the lavender plant and placed them in a laboratory flask with a capacity of (250) ml. They were washed with water and liquid soap, then placed under running tap water for one hour to remove the dust from them After that, it was transferred to the stratified air flow table to perform the sterilization process, where it was surface sterilized with different concentrations of sodium hypo chlorate (NaOCl) for the purpose of sterilizing plant parts and concentrations (0, 2, 4, 6) for a period of (5, 10, 15, 20). minutes and then transferred to a 250 ml glass container containing ethyl alcohol at a concentration of (70%) for a minute and then washed with sterile distilled water three times to remove the remains of sterilizing materials. After that, they were placed in Petri dishes that had previously been sterilized with alcohol and burned by flame, and they were divided into As in Figure (1), it was planted on medium (7) and incubated in the growth room at a temperature of (25 ± 2) and a light intensity of (1000) lux (16) hours of light followed by (8) hours of darkness in succession. The results were taken two weeks after planting.

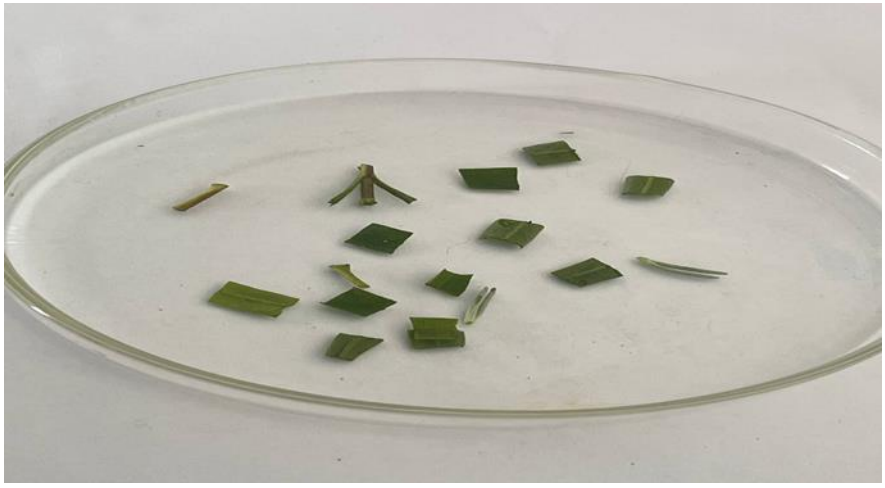

Figure 1: Plant parts prepared for cultivation

(4.49) grams of the ready-made powder for the nutrient medium were weighed to prepare a liter of growing food plant parts, and agar was added to it as a solidifying substance for the nutrient media in an amount of (7 g/l) after adding sucrose (3%), BA and 2.4-D according to the requirements of the experiment and was modified. The medium was reduced to (6.5) by adding hydrochloric acid and (HCI) or sodium hydroxide solution (NaOH), and cooking the medium by placing it on the Hot plate magnetic stirrer, then pouring it into test tubes at a rate of (10 ml) for each tube and after closing

them. Place it tightly in an autoclave at a temperature of (121°C). Pressure (1.04 kg cm) was applied for a period of (15 minutes), then it was taken out of the incubator and left to cool and the medium solidified at room temperature, thus becoming ready for planting. The experiment was conducted to determine the effect of 2,4-D and BA and their interaction in growing lavender plant parts in nutrient media. The plant parts were grown on sterile media (13) provided with different concentrations of the growth regulator auxin (NAA) at concentrations (0.0, 0.5, 1, and 1. 5 and 2) mg L^{-1} and cytokine (BA) in concentrations (0.0, 1, 1.5, 2.5 and 2) mg L^{-1} and their interactions in the propagation of lavender plants ex vivo and their effect on the production of vegetative shoots and the emergence of a vegetative and root group, and five replicates.

RESULTS AND DISCUSSION

3-1 Effect of 2,4-D and BA on the percentage of callus induction from the vegetative part of a plant Lavender 45 days after planting:

The results in Table (1) show that there was a significant effect of the growth regulator D (2,4) in increasing the percentage rate of callus induction from the growing top of the lavender plant after six weeks of planting, as the concentration of 1 mg per liter was significantly superior to all treatments by giving it the highest percentage of (2.4). (79.2%) followed by a concentration of 1.5 mg .L^{-1} (61.0%), while the comparison percentage was at the lowest value of (0.0%). The results of the same table also showed that the use of the growth regulator BA had a significant effect in increasing the percentage of callus induction, as the two concentrations exceeded 1 mg L^{-1} and 2 mg. L^{-1} over the rest of the concentrations, giving them the highest percentage of callus induction, amounting to (49.2 and 47.6%), respectively, while the control treatment gave the lowest percentage of callus induction, amounting to (37.0%). The results of the same table showed that there was a significant effect of the interaction between the growth regulators (2,4-D-D) and (AB) in increasing the percentage of callus induction, as most combinations gave concentrations of (2,4-D) (mg L-1) and BA) (mg L-1). 1 - The highest values reached (95.0%), while the comparison treatment did not give, and cytokines are used in low concentrations to produce physiological effects in the cultivated plant part, and in balance with auxins, they help in inducing callus (8). A number of researchers have indicated the possibility of inducing callus depending on the plant part and the growth regulators added to the nutrient medium, and the balanced combination increases the strength of callus stimulation (9). It has become clear from this result that the relationship between the concentration and quality of the growth regulator and the ratio between them is the main factor in the process of inducing callus from Plant tissues (10). This may be due to the variation in the physiological response and physiological age present in the plant parts and that the response to callus formation varies from one plant part to another. Also, the type of cells in these parts and there are plant parts that have given rise to callus and the reason is due to its recentness (Juvenality). These parts, because their meristematic cells are active and their internal content of growth regulators increases (11), The reason for the induction of callus may be due to a balance between auxins and cytokines added to the culture medium with the hormones that are present inside the cells, which work together to elongate the longitudinal axis of the cells and also encourage cell division (12). It has been shown that the process of callus induction, differentiation, and then its differentiation is affected These results are consistent with (13) what was indicated when they induced lavender callus by adding different levels of BAP and D2, 4, as it gave the best response using the addition of regulators, and the lavender callus induction rate was 67%. Likewise, (14) indicated the induction of callus from lavender using different concentrations of NAA and BAP. They confirmed that high levels of NAA and low levels of BAP produced good callus. Lavender callus was obtained by growing it in a medium supplemented with IAA and Kin. With varying concentrations of both types of growth regulators(15), abundant greenish-brown lavender callus devoid of phenolic compounds in the media was induced by the addition of D-2,4, BAP, and GA3 using different levels of them (16).

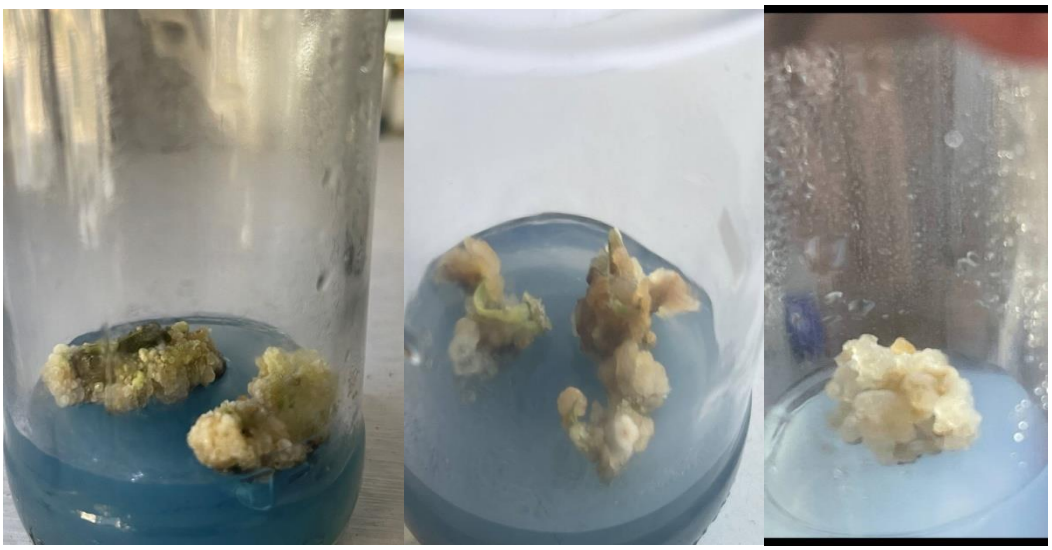

Figure 2: Callus induction after 45 days of planting the vegetative part of the lavender plant in the nutrient medium prepared with a concentration of 1.

Table 1: Callus induction after 45 days of planting the lavender plant part in the nutrient medium prepared with a concentration of 1

Average 2,4-D	BA mg L^{-1}					2,4-D mg L^{-1}
	2	1.5	1	5.0	0	
0.0	0.0	0.0	0.0	0.0	0.0	0
46.6	42.0	42.0	54.0	47.0	48.0	0.5
79.2	61.0	62.0	95.0	90.0	88.0	1
61.0	55.0	50.0	63.0	67.0	70.0	1.5
30.8	27.0	28.0	34.0	33.0	32.0	2
	37.0	36.4	49.2	47.4	47.6	Average BA
	Interaction=1.467		BA=0.656	2,4-D=0.656	L.S.D 0.05	

3-2 The effect of NAA and BA, or their interaction, on the number of branches (plant branches) 30 days after planting:

The results of Table (2) showed that there were significant differences between the concentrations of NAA, as all the concentrations of NAA used were significantly superior to the comparison treatment. The concentration of 0.5 mg L^{-1} gave an increase in the average number of branches, amounting to 3.18 plant branches, which was significantly superior to the comparison treatment, 2.01 plant branches. . The reason for the increase in the rate of the number of vegetative branches may be due to the synergistic action between cytokine and auxin in encouraging vegetative growth at higher concentrations(17) ,We note from the same table that there is a significant effect of the growth regulator BA, as the concentration of 2.5 mg/L was significantly superior to some concentrations in the number of branches by giving it the highest rate of 3.30 branches per plant part. The reason for the increase in the rate of branches when adding BA to the nutrient medium may be due to its role in canceling the apical dominance and liberating the lateral shoots, thus increasing the rate of the number of vegetative branches. The effectiveness of BA in multiplying the branches is due to the side chain containing three double bonds (18). This is consistent with What was mentioned (19) The role played by BA at concentrations appropriate for tissue culture works to break the apical dominance and creates areas of attraction source-sink relation in the buds and stimulates the transfer of nutrients and other growth materials to them, which results in the stimulation of vegetative buds and their growth. As for the effect of NAA, adding it in appropriate concentrations causes An increase in the number of branches. The reason for the decrease in vegetative branches with high concentrations of added NAA may be due to its increased accumulation in the cultivated plant part. Therefore, it leads to an imbalance in the hormonal balance and thus inhibits branching as a result of the process of antagonism with BA, and then reduces the side branches as a result of strengthening apical dominance(20). Auxin prevents the occurrence of vascular communication between the vascular tissues of the axillary buds and the vascular tissues of the stem, which leads to no or limited passage of nutrients from the stem tissues to the buds and thus weakening their growth (21) (22),It has become clear from the results shown that the number of plants per lavender plant increases with increasing NAA concentrations. And BA and Kin may be due to the role of cytokines as plant growth regulators that stimulate bud growth by stimulating cell division and reducing apical dominance (23), as (24) indicated that using concentrations of IAA (0.4) and BA (0.8) mg L-1 was the best. To obtain a number of lavender plants, After using two groups of auxins and cytokines (IAA, BA, Kin, and IBA), which encourage the growth of the pre-existing meristem easily into buds with increased cell division. It was observed that the number of branches of the lavender plant grew and increased after 20 days of planting it in the agricultural medium, after digging up the buds, and the buds were restored. Cultivating them on the same medium to obtain a greater number of multiple shoots than using different concentrations of NAA, BA, kin, and 2-4,D in appropriate *concentrations (25).*

Table 2: Effect of NAA and BA and their interaction on the number of branches 30 days after planting

Average NAA Mg L^{-1}						BA / NAA
	2.5	2	1.5	1	0	
2.01	4.23	2.22	1.24	2.15	0.22	0
3.18	4.2	4.3	5.4	1.68	0.32	0.5

2.37	2.88	2.98	2.92	2.7	0.37	1
1.38	2.19	2.37	1.12	1.11	0.11	1.5
2.09	3.01	3.14	2.24	2.04	0.02	2
	3.30	3.00	2.58	1.93	0.28	BA Average Mg L^{-1}
	Interaction=0.223			BA=0.099	NAA=0.099	L.S.D 0.05

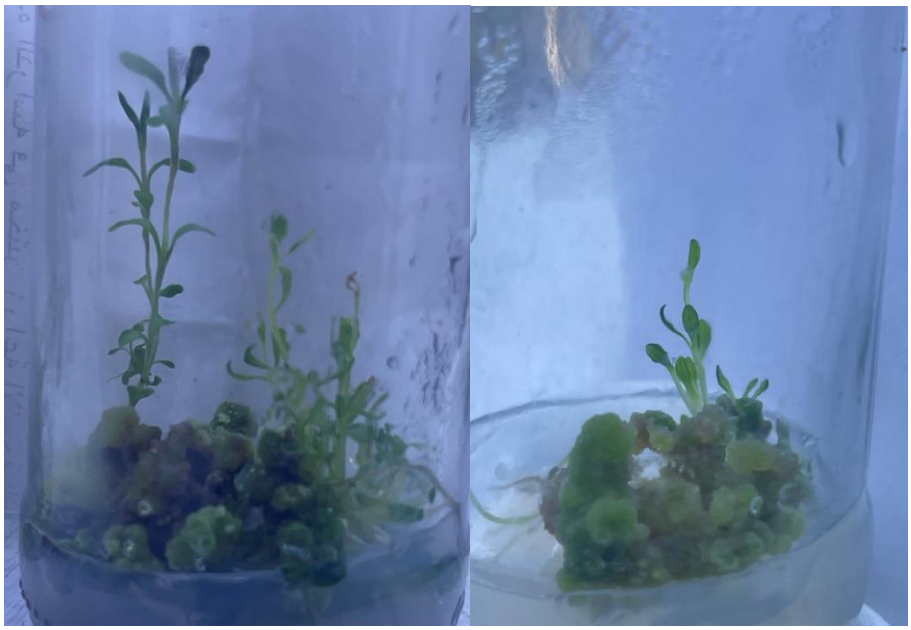

Figure (2): The effect of NAA and BA and their after planting interaction on the number of branches 30 days

CONCLUSIONS

1- The dual interaction between the growth regulators(2,4-D)and(AB) increased the percentage of callus induction, as most combinations of concentrations of (2,4-D) mg L^{-1} and(BA) mg L^{-1}gave the highest values. 95.0%, while the comparison treatment did not give.

2- The binary interaction between (NAA) and (BA)at a concentration of 0.5 mg L^{-1} and 1.5 mg L^{-1} gave the highest significant differences in the multiplication of vegetative branches from cultivated plant cuttings.

References

Al-Gburi,O.B.A.(2021).Induction and detection of gene expression in Abelmoschus esculentus tissue cultures toward drought tolerance.PH.D.thesis/Department of Biology/college of Science/Tikrit University/Iraq.

Al-Garallaa,K.(2021).Phytochemical and biological strategies to improve essential oil content in lavender.Ph.D.thesis,College of Agriculture and life sciences/Mississippi state University/Mississippi,USA.

Al-Sumaidaie, Kazem Muhammad Ibrahim (2015) "Applications in Plant Biotechnology." Ministry of Higher Education and Scientific Research. Part One, Al-Nahrain University, Dar Al-Kutub for Printing and Publishing, Iraq.

Al-Rifai, Ab; .del-Rahim Tawfiq and Al-Shoubaki, ; Samir Abdel-Razzaq. (2007). Plant tissue culture and plant micropropagation. Egyptian Library for Printing, Publishing and Distribution - first edition, Alexandria - Egypt.

Wesolowska, A. ; Jadcak, p.,; Kulp,a and d.przewodowskiw.(2019).Gaschromatograaphy Mass spectrometry (Gc-ms) analysis of essential oils from agwps and aurps elicted lavandula angustifolia in vitro cultur Molecules 24(3):606.

RODRIGUES, DB.;, RADKE, AK. SOMMER LR ROSA, DSb., SCHUCH, m. and W AsSLS, AM.(2020). quality of light and indolbutiric acid in vitro rooting of lavender ornamental Horticulture 26 :89-94.

Murashige T,.(1978). Principles of rapid propagation. In: K. W. Hughes et al. (Eds.), Propagation of higher plants through tissue culture. U.S. Dept. of Energy. Washington D.C., pp. 14-Morel G, 1960 - Production virus - free Cymbidium. Am. Orchid Soc. Bull, 29: 495-497.

Evans, D.A. (1989). Somaclonal Variation - Genetic basis and breeding a application . Trends Genet .95:46-50.

Gadomosi, A.E. and B, Shaibu. (2013). In fluenco of the phytohormones on the in vitro regeneration in Sema alata (Linn). Acad. J.Biotech.,1(3):41-45.

Kumar A, Sood A, P alni LMS, and AK.Gupta (1999). ln vitro propagation of Gladiolus hybridus Hort.:Synergistic effect of heat shock and sucrose on morphogenesis.Plant cell Tiss. Org. Cult. 57:105-112.

Dodds, J.H. and Roberts, W. (1985). Experiments in Plant Tissue Culture . Cambridge University. Press. UK England.

Pathania, N.S, Misra, R.L . and S.P.S. Raghave, (2001).Precocious shoot proliferation and microcorm production in gladiolus through tissue culture.J. Ornam. Hort. (NS),4:69-73.

Tan Nhut D.,Teixeira Da Silva J. A., Huyen and P. X., Paek. (2004).The importance of explant source on re-generation and micropropagation of Gladiolus by liquid shake culture. Scientia Horticulturae, 102: 407-414.

Taiz, L. and Zeiger, E. (2013) Fisiologia Vegetal. 5th Edition, Artmed, Porto Alegre, 918 p

Bona,C.M.;D.Santos,G.and A.Biasi,Luiz,(2012).Lavandula calli induction, growth curve and cell suspension formation.v.7,n1, p.17-23.

Luoya.((2018.Tissue culture of Lavandula angustifolia L.AIP Publishing.College of horticulture.Sichuan agricultural university.china.978-0-7354-1807-3.

George, E. F.; M. A. Hall and Klerk, G. D. (2008). Plant propagation by tissue culture 3rd edition. Published by spring, P.: 1- 479.

KOEFENDER,J;MANFIO,CE;CAMER,JN;SCHOFFEL,A;GOLLE,DP. 2021.Micropropagation of lavender: a protocol for production of plantlets.Horticultural Brasileira 39: 404-410.

Lilien-Kipnis, H. and M. Kochba. (1987). Mass propagation of new gladiolus hybrids. Acta. Hort.,212:631-638.

Bruyn M.H., D.I. Ferreira and M.H. Debruyn (1992). In Vitro corm production of Gladiolous dalenii and G.tristis. Plant cell Tissue and Organ Culture,31:2,123-128.

Jager,A.K; BG. Mcalister ;J. V. Staten and S.J. Van (1998) In vitro culture of Gladiolus careens African of Botany, 642,146-South

Mohsen, Muhammad Mahdi. (2009) Propagation of Gladiolus bulbs (Gladiolus sp.) using tissue culture technology. Master's thesis, Al-Musayyib Technical College, Al-Furat Al-Awsat Technical University, Republic of Iraq.

Kim, K W., MS. Kang, and DH. Goo (1991). The external and histological characteristics of organogenesis from Gladiolus callus Korean Soc. Hort. Sci. 32:124-129.

Goncalves Sandra;Romano Anabela (2013).In Vitro Culture Of Lavenders(Lavandula app.)and the production of secondary metabolites.Bioteechnology Advances 31:166-174.

El-sharnouby,Mohamed,Ehab(2022).Torrance limits of lavender plant (lavandula angustifola mill)tissue culture in response to ablotlc stress..college of sciene,taif University ,taif21944,Saudi Arabia,J.pak.Bot54(6):2063-2068.

Leelavathi.Dr.D.(2010).Ex-Situ conservation of lavandula angustifolia using in vitro techniques .mes college ,mallesw aram,Bangalore 560-003.