Effect of Growth Regulators and BA on lavender Callus Induction In vitro

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Abstract

This study was conducted in the plant tissue culture laboratory of the Department of Plant Production Technical / Al-Musayyab Technical College/Al-Furat Al-Awsat Technical University from 1/9/2023 to 1/5/2024 to study the effect of some plant growth regulators: Sterilization experiment with Naocl sodium hypochlorate at concentrations of(0, 2, 4, 6)% for (5, 10, 15, 20) minutes and auxin 2,4-D (2,4-Dichlorophenoxyacetic acid)at concentrations (0.0, 0.5, 1, 1.5, 2) mg L^{-1} and cytokinin BA (Benzyladenine) at concentrations (0.0, 0.5, 1, 1.5, 2) mg L^{-1} and the experiment on the fersh weight of callus with different auxin 2,4-D(2,4-Dichlorophenoxyacetic acid) in concentrations (0.0, 0.5, 1, 1.5, 2) mg L^{-1} and cytokinin BA(Benzyladenine) at concentrations (0.0, 0.5, 1, 1.5, 2) mg L^{-1} and their interactions in the propagation of lavender plants in vitro and their effect on callus induction and vegetative and root and shoot generation. The known nutrient medium was used (Murashig and Skoog, 1962). This study was designed according to a completely randomized design(CR) with ten replications, then the means were compared using at least significant difference LSD under the probability level (0.05).

Keywords: auxin, cytokinin, callus ,induction , fresh and dry weight of callus.

1.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, and cosmetics[1]. The original homeland of the lavender plant is in the Mediterranean regio[2]. While propagation through seeds is slow, vegetative reproduction is necessary to produce genetically homogeneous individuals, and the rootstock is ineffective[3]. Using the micro propagation technique allows the production of large numbers of plants within a short period of time, making it possible to propagate plants that are difficult to propagate using traditional methods[4]. 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 and dry weight), the effect of growth regulators on shoot formation and root formation, the production of lavender in vitro.

2.Materials and methods

the leaves taken and the growing top from lavender plant with a length of (1-5.0) cm, They were washed with water and liquid soap, then placed under running tap water for one hour to remove any dirt. After that, they samples were transferred to a laminar flow hood for sterilization process, they were surface- sterilized using with different

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concentrations of... Sodium hypochlorite (NaOCl) for varying durations to sterilize the plant parts and concentrations (0, 2, 4, 6) for (5, 10, 15, 20) minutes , Then the samples were then transferred to a 250 ml glass container containing (70%) ethanol for one minute a washed with sterile distilled water three times to remove any residual sterilizing agents. Subsequently, they were placed in Petri dishes that had been previously been sterilized with alcohol and flame. The pollution rate was extracted based on the pollution figures. Then the samples were then divided, and planted on medium2,4-D in concentrations (0.0, 0.5, 1, 1.5, 2) mg L^{-1} and cytokinin BA at concentrations (0.0, 0.5, 1, 1.5, 2) mg L^{-1} (10).They were incubated in the growth room at a temperature of 25 ± 2 with a light intensity of 1000 lux for 16 hours followed by 8 hours of darkness alternately. The results were observed two weeks after planting. (4.49) gm of the ready-made culture medium powder were weighed to prepare one liter for plant tissue culture , and 7gm of agar per liter were added as a solidifying agent , along with the addition of 3% sucrose, BA, and 2,4-D according to the requirements of the experiment and was modified. PH of medium is reduced to (6.5) by adding hydrochloric acid and HCI or sodium hydroxide solution NaOH, and the medium by placing it on the Hot plate magnetic stirrer, Then it was poured into test tubes at a rate of 10 ml for each tube, and after closing them tightly, it was placed in the autoclave at a temperature of 121°C and a pressure of 1.04 k/g^2 cm for a 15 minutes. Then it was taken out of the autoclave and left to cool and the medium would solidify at room temperature. Thus, it is 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 [5]provided with different concentrations of growth regulators 2,4-D (0, 50, 1, 1.5, and 2) mg L^{-1} and (BA 0, 0.5, 1, 1.5, and 2) mg L^{-1} using five replicates.

3.Result and Discussion

3.1-The effect of sodium hypochlorite concentrations and the sterilization period on the percentage of contamination of the vegetative part of the lavender plant 15 days after planting:

The results in Table (1) indicate the effect of sodium hypochlorite concentrations and the sterilization period in reducing the contamination rate of plant parts. The pollution rate was extracted based on the pollution figures. The contamination rate reached 100% in the neutralizer treatment, and the contamination rate decreased with increasing the sterilization period, reaching 60% at a duration of 15 minutes. On the other hand, the concentration 4% gave the lowest contamination rate of 24%, and the effect of sodium hypochlorite concentrations and time duration was significant. The contamination rate decreased to 20% with a duration of 15 minutes at a concentration of 4%. The growth of was good, but the interaction between the concentration 6% and the duration 20 minutes had the lowest contamination percentage, amounting to 45%, but it led to the death of the plant part. Some sterilizers have a fatal effect on cell division and restrict the growth and development of the plant part. Therefore, the appropriate concentration, formulations, and duration of exposure to the sterilizer are essential for the success of cultivation in the laboratory [6]. prefer to use sodium hypochlorite in sterilization due to its efficiency and not harming the plant part. The reason for this is the percentage of the active

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ingredient in the sterilization agent NAOCl, which is used at levels in the study and that it may be non-toxic to the plant part without causing harm to the components of the part. Plant sterilization in the laboratory is an essential step for plant tissue cultivation, and the final results of cultivation in the laboratory depend directly on the efficiency of sterilization [7]. Sterilization of lavender plants depends on the survival rate and sterilization rate. The longer of sterilization time, gave lower the contamination rate. However, the survival rate was related to the genotype, type of transplant, and age of the transplant. The results showed that the plants had a relatively high survival rate and a low contamination rate when sterilization was carried out over a period of 13 minutes at a concentration of 0.1 mgL^{-1}, and the sterilizing substance was NAOCL, and sterilization over a period ranging between (6-8) minutes was the best in sterilization, which is due to the genetic differences of the lavender plant[8].

Table (1): The effect of NAOCL concentrations and the sterilization period on the percentage of contribution to combating corruption. Groing tops and leaves of planted lavender 15 days.

Concentrations rate **Mg l^{-1}**	**Time**				**Concentrations** **percentage**
	20 Minute	**15 minute**	**10 minute**	**5 minute**	
100	100	100	100	100	**0 Mg l^{-1}**
82.25	70.00	77.00	82.00	100	**2 Mg l^{-1}**
24.00	27.00	20.00	24.00	25.00	**4 Mg l^{-1}**
43.25	45.00	43.00	44.00	41.00	**6 Mg l^{-1}**
62.37	60.5	60.00	62.50	66.50	**Time rate minute**
	Interaction 1.663=		time 0.832=	Concentrations 0.832=	**L.S.D 0.05**

3.2-Effect of 2,4-D and BA on the percentage of callus induction from the vegetative part of a Lavender plant after 6 weeks from planting

The results in Table (2) show that there was a significant effect of the growth regulator 2,4-D(2,4-Dichlorophenoxyacetic acid) in increasing the percentage rate of callus induction from the growing top of the lavender

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plant after six weeks of planting. The concentration of 1 mg L^{-1} significantly exceeded all treatments by giving the highest percentage of 79.2%, followed by a concentration of 1.5 mg L^{-1} at 61.0%, while the control 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. The two concentrations of 1 and 2 mg L^{-1} exceeded the rest of the concentrations, giving the highest percentages of callus induction at 49.2% and 47.6%, respectively, while the control treatment gave the lowest percentage of callus induction at 37.0%.The results of the same table indicated that there was a significant effect of the interaction between the growth regulators 2,4-D(2,4-Dichlorophenoxyacetic acid) and BA(Benzyladenine) in increasing the percentage of callus induction. Most combinations of 2,4-D(2,4-Dichlorophenoxyaceticacid) and BA(Benzyladenine) mg L^{-1} gave the highest values, reaching 95.0%, while the control treatment did not result in any callus induction. Previous studies have shown that the ability of plant parts to form callus increases when the nutrient medium is prepared with growth regulators such as cytokinins and auxins. Their interaction at different concentrations is important for callus formation and growth, to achieve optimal compatibility in the internal hormonal status of the plant part compared to using them individually[9]and[10]. Cytokinins are used in low concentrations to produce physiological effects in the cultivated plant part, and in balance with auxins, they help in inducing callus[11]. This result indicates that the relationship between the concentration and type of growth regulator, and the ratio between them, is the main factor in the process of callus induction from plant tissues[12].This may be due to the variation in physiological response and the physiological age present in plant parts, as the response to callus formation varies from one plant part to another. Additionally, the type of cells in these parts and the newness (juvenility) of these parts contribute to callus formation due to the activity of their meristematic cells and an increase in their internal content of growth regulators[13].Researchers have indicated that the interaction between auxins and cytokinins and their compatibility leads to callus induction, and adding cytokinins with auxin helps in obtaining more solid callus[14]. The process of callus induction, differentiation, and subsequent development is influenced by several factors, including the components of the culture medium, the type and concentration of growth regulators, the physiological state of the mother plant, the source of the plant part, the genetic composition of the plant, and environmental conditions[15].Most researchers consider the genetic composition and nutritional components to be the main sources of differences in response when grown outside the living body[16]. These results are consistent with what researchers found when they induced lavender callus by adding different levels of BAP and 2,4-D, which gave the best response using the addition of regulators, with the rate of lavender callus induction being 67%[17].

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Table 2: Callus induction mg L^{-1} after 45 days of planting the lavender plant part tops in the nutrient medium prepared with a concentration

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

3.3 -Effect of BA and 2,4-D and their interaction on the fresh weight (mg) of callus induced from the vegetative part of lavender after 42 days of planting:

The results in Table (3) show that the concentrations of 2,4-D(2,4-Dichlorophenoxyaceticacid) mg L^{-1} had a significant effect on increasing the average fresh weight of callus induced from the vegetative part of the lavender plant after 42 days of planting. The concentration of 2 mg L^{-1} of 2,4-D(2,4-Dichlorophenoxyaceticacid) gave the highest average fresh weight of 0.85 mg L^{-1}, while the control treatment gave the lowest fresh weight of 0.01 mg L^{-1}. The concentration of 2 mg/$L^{-1}$of BA(Benzyladenine) also significantly outperformed the other concentrations, giving the highest average fresh weight of 0.55 mg L^{-1}, while the control treatment gave the lowest fresh weight of callus at 0.38 mg L^{-1}. The results of the same table also indicated a significant interaction between the growth regulators 2,4-D(2,4-Dichlorophenoxyaceticacid) and BA(Benzyladenine) in increasing the fresh weight of the induced callus, as the combination of 2,4-D at a concentration of 2

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mg L^{-1}+ BA(Benzyladenine) at a concentration of 2 mg L^{-1} gave the highest fresh weight of the callus at 1.2 mg L^{-1}, while the control treatment did not produce any fresh weight of callus due to its lack of response to callus formation. The reason for the increase in the fresh weight of the callus is attributed to the addition of auxin, which stimulates the softening of the cell wall by breaking and reforming cell wall bonds under turgor pressure, thus increasing cell size and expansion [18].The effect of cytokinins in inducing callus may be due to their role in creating sinks that accelerate the transfer of water and nutrients, leading to the stimulation of division and growth of cells cultured in vitro, thereby increasing the callus weight. This effect is observed at optimal concentrations, while higher concentrations may be toxic [19].The increase in the fresh weight of the callus is attributed to the addition of an appropriate concentration of BA (benzyladenine), which balances the positive and negative charges on the cell membrane, enhancing the absorption of other growth regulators and leading to increased biosynthesis, protein synthesis, and cell division, thereby increasing fresh weight [20].The presence of cytokinin in the medium is important for promoting callus growth, leading to an increase in fresh weight due to the balance between auxins and cytokinins and the internal cell balance that encourages cell division and elongation. Conversely, high concentrations may disrupt this balance, reducing fresh weight [21]. These results are consistent with findings from researchers who used BA with NAA or 2,4-D in the culture medium to measure the fresh weight of callus in lavender plants[22].

Table (3): Effect of 2,4-D and BA and their interaction on the fresh weight (mg) of callus induced from the growing shoot after 42 days of planting

Rate 2,4-D mg L^{-1}	**2 mg L^{-1}**	**1.5 mg L^{-1}**	**1 mg L^{-1}**	**0.5 mg L^{-1}**	**0 mg L^{-1}**	**BA mg L^{-1} / 2,4-D mg L^{-1}**
0.01	0.08	0.00	0.00	0.00	0.00	**0 mg L^{-1}**
0.20	0.3	0.22	0.61	0.51	0.2	**0.5 mg L^{-1}**
0.85	1.2	0.7	0.8	1.04	0.52	**1 mg L^{-1}**
0.71	0.7	0.79	0.8	0.66	0.6	**1.5 mg L^{-1}**
0.61	0.5	0.57	0.61	0.79	0.6	**2 mg L^{-1}**
	0.55	0.45	0.47	0.52	0.38	**Rate BA mg L^{-1}**
	= Interaction 0.453		BA= 0.203		2,4-D =0.203	L.S.D 0.05

Consolation

The results showed that the contamination rate reached (100%) in the comparison treatment, and the contamination rate decreased with increasing sterilization duration. The contamination rate reached (45%) at the duration (20) minutes, at the concentration

(6%). On the other hand, the concentration was (4%) less. The contamination rate reached (24%), while in the stage of callus induction, the concentration of (1) mg L^{-1} was significantly superior to all treatments by giving it the highest rate of (79.2%) followed by 1.5 mg L^{-1}, which reached (0.61), while the The comparison percentage at the lowest value reached (2.9%), while the growth regulator BA(Benzyladenine) had a significant effect in increasing the percentage of callus induction, as the two concentrations (1) mg L^{-1} and (1.5) mg L^{-1} outperformed the result of the concentrations by giving them the highest percentage. The percentage of callus induction reached (49.2 and 47.6%), respectively, while the comparison treatment did not give any results. The highest concentration of fresh callus weight reached 1.2 mg from using the combinteration of 1 mg L^{-1} and BA (Benzyladenine) at a concentration of 2 mg L^{-1},

Meanwhile, the control treatment did not yield any fresh callus weight .

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