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المجلة العراقية لبحوث السوق
وحماية المستهلك

THE ROLE OF PLANT GROWTH REGULATORS IN THE INDUCTION OF CALLUS TREATED WITH HARMEL SEEDS EXTRACT AND THE REGENERATION AND ROOTING OF VEGETATIVE GROWTHS OF BARLEY VARIETIES IN VITRO

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ABSTRACT

An experiment was conducted in the Plant Tissue Culture Laboratory at the College of Agricultural Engineering Sciences, University of Baghdad during the years 2022-2023 using Plant Tissue Culture Technique. The variables three varieties of barley: Ebaa265, Ebaa99 and Bouhoth 244; 2,4-D (2,4-Dichlorophenoxyacetic acid) at concentrations: (0,1.5,3 and 4.5) mg.L^{-1} for callus inducation; BA (Benzyl Adenine) at concentrations: (0,1,2, and 3) mg.L^{-1} for regeneration and IBA (Indole Butyric Acid) at concentrations :(0,1,2, and 3) mg.L $^{-1}$ for rooting ,using MS (Murashige and Skoog) media. Measurements has taken on average of the weight wet and dry of the callus and number and length shoot and root. Done results revealed significant differences in all treatment. Thus, obtaining plants with a higher quantity and quality of food that meets the consumer's need and increases the marketing value of the varieties.

Key words: 2, 4-D, BA, IBA, Plant Tissue Culture, consumer.

دور منظمات النمو النباتية في إستحثاث الكالس المعامل بمستخلص بذور الحرمل وإعادة التمايز وتجذير النمو الخضرية في أصناف من الشعير خارج الجسم الحي

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الخلاصة

أجريت تجربة في مختبر زراعة الأنسجة النباتية في كلية علوم الهندسة الزراعية جامعة بغداد خلال السنتين 2022-2023 وظفت خلالها تقنية زراعة الأنسجة النباتية لإكثار ثلاثة أصناف من الشعير: إباء265، وإباء99، وبحوث 244. أستعمل 2,4- D (2،4 حامض ثنائي كلوروفينوكسي أسيتيك) بالتركيز: (3،1.5،0 ،4.5) ملغم.لتر$^{-1}$ لاستحثاث الكالس و BA (بنزيل أدنين) بالتركيز (3،2،1،0) ملغم.لتر$^{-1}$ للتمايز و IBA (حامض الإندول بيوتريك) بالتركيز: (3،2،1،0) ملغم.لتر$^{-1}$ للتجذير واستعمل الوسط الغذائي MS (Murashige وSkoog). تم أخذ القياسات لمتوسط الوزن الرطب والجاف للكالس وعدد وطول الساق والجذور. أظهرت النتائج وجود فروق معنوية في جميع المعاملات. بالتالي الحصول على نباتات ذات كمية ونوعية غذائية أعلى تسد حاجة المستهلك، وترفع من القيمة التسويقية للأصناف.

الكلمات المفتاحية: 2،4-D ، BA، IBA ، زراعة الأنسجة النباتية، المستهلك.

[*] **This article is taken from the doctoral dissertation of the first researcher.**

INTRODUCATION

Recent medical studies have confirmed that using barley in the diet helps lower the level of cholesterol in the blood, which benefits people with diabetes and has an effect in treating cancer and delaying aging because barley contains antioxidants such as vitamins A and E **(Eman & Hadi,2023; Chikako *et al*., 2019; Attila, 2019).**

The idea of transplanting cells, tissues and organs of plants was based on the idea of totipotency, as this idea suggests that every living cell sepaaveraged from a living organism has the ability to divide and develop in isolation from the living organism from which it is sepaaveraged if the conditions for division, development and growth are provided for it **(Baday, 2020; Cox,2018).**

Plant tissue culture is an essential component of plant biotechnology because it provides a means of propagating and forming new plants from genetically modified cells and the resulting plant can be easily cloned in sterile conditions **(Al-Jubori & Al-Amery, 2022; Loyola-Vargas & Ochoa-Alejo, 2018).**

The callus produced through plant tissue culture technology is considered a good raw material for research on genetic variation because the callus is composed of a mass of undifferentiated meristematic cells, which in themselves are susceptible to genetic variation **(Mohammed *et al*., 2019; Davani *et al*.,2017)**.

Auxins are used to encourage callus growth as well as organ growth in tissue cultures. They also work to regulate differentiation processes, especially when added with cytokinins. Auxins stimulate cell division and the start of the formation of meristematic centers in tissue cultures, which can be discovered later **(Toma,2022; Salman,2014).** Harmel is a plant with medicinal uses, as its seeds contain three alkaloids: Harmaline, Harmine, and Harmalol **(Shahrajabian *et al*., 2021)**.**Al-qubbi & Tika (2014)** revealed that the behavior of chromosomes became abnormal in samples treated with aqueous Harmel extract at a concentration of 0.05 also, abnormalities in the genetic material, chromosomal fractures, and a change in the location of the genetic material were observed, in addition to distortions in the apparent appearance of the dividing cells in peas.

The presence of these meristematic areas encourages the speed of growth as they centers for manufacturing IAA, as auxins work to acceleaverage the growth of tissues by expanding and dividing cells. This is done by pumping hydrogen ions through the cell walls, as the binding of auxin to the cell walls breaks down the lipids that make them up, and thus the acidity and elasticity of the cell walls increases and the average of potassium ion absorption increases, which leads to neutralize the hydrogen ion, this results in a decrease in the value of the osmotic pressure of the cell and an increase in its ability to absorb water and the cell expands **(Azizan *et al*., 2023; Ling *et al*., 2013).**

The release of the hydrogen ion leads to an increase in the acidity of the external environment and the occurrence of ion exchange. In this way, auxin works indirectly to stimulate the synthesis of the enzyme ATPes, which is located in the cell membrane and is

responsible for Transporting hydrogen and hydroxyl ions to and from the cell, increasing the permeability of the walls to other ions **(Al-Bayati & Al-Juboori, 2023)**.

Cytokinin stimulates the production of nucleic acids as a result of activating and stimulating the enzyme that transfers nucleic acids (Synthetase-T-RNA), and this stimulation is reduced by the enzyme Ribonuclease. This shows that cytokinin stimulates the production of proteins and nucleic acids because of stimulating the activity and effectiveness of the genes responsible for the formation of enzymes, especially the nitaverage enzyme nitaverage reductase **(Al-Amery & Al-jubori, 2020)**.

Cytokinins are specialized in cell division and increasing their growth. They also encourage vegetative growth, increase lateral growth, and break apical dominance. They are used with auxins to form callus when used in the appropriate concentration, as they work to increase the number during plant propagation, help regulate cell division, stimulate the generation of adventitious and subsidiary growth, regulate differentiation, and inhibit root formation. RNA synthesis and stimulating the work of proteins and enzymes **(Baday, 2019).**
Hairuddin *et al.* (2023) reported that adding BA at a concentration of 1.5 mg.L^{-1} in interaction with NAA was the best in re-differentiation and organ formation of yellow maize embryos, as the weight of the leaves reached 100 mg and their height was 11 cm.

Auxins effectively affect the growth of roots, especially when added in appropriate concentrations, while high concentrations cause inhibition of their growth. The process of the emergence of adventitious roots outside the living body begins with the loss of cell differentiation and the emergence of meristematic areas in the stem. Then these cells multiply and become swollen and spherical in shape, and the cells continue to multiply and divide. To form areas called root meristems, then the cells in the basal part of the root meristem area elongate to produce newly formed roots **(Salman *et al*.,2022)**.
Ayudhaya *et al.* (2023) showed that when using 2,4-D at a concentration of (0,2,4,6) mg.L^{-1}, the highest average wet and dry weights of rice callus reached 31 and 15 mg, respectively, at a concentration of 2 mg. L^{-1}. **Xu *et al.* (2022)** 2,4-D was adopted at a concentration of 2.5 mg. L^{-1} and BA at a concentration of 0.1 mg. L^{-1} in the induction of callus from barley grains. This study aims to evaluate the response of three barley varieties to plant growth regulators by determining the concentration of 2,4-D which stimulates callus formation, and determining the concentration of BA and IBA in the re-differentiation of shoots and roots.

MATERIALS AND METHODS

Three varieties of barley, Ebaa265, Ebaa99, and Bouhoth244, obtained from the General Company for Seed Testing and Certification, were used in this study (**Hosseini *et al*., 2014)** .The seeds were sterilized with 3% sodium hypochlorite for 15 minutes, and the sterilized seeds were washed with sterile distilled water three times to remove traces of the sterile material in the stratification table **(Toma, 2022; Al-Juboori, *et al*., 2019 ; Duan *et al*.,2019)**

In this study, ready-made MS medium weighing 4.9 $g.L^{-1}$ was used at all stages of cultivation, and plant growth regulators, 2,4-D were added at concentrations of (0, 1.5, 3, and 4.5) $mg.L^{-1}$ for callus induction **Najm & Hamza, 2023)**, Harmal seed extract was prepared using the method of (**Singh, 2008)**. and BA at concentrations of (0, 1, 2, and 3) mg. L^{-1} In reforming vegetative growths and IBA at concentrations of (0, 1, 2, and 3) $mg.L^{-1}$ in rooting growths, adjust the pH of the nutrient medium to 5.8, and sterilize it with a steam sterilizer (Autoclave) for 20 minutes, at a temperature of 121°C, and a pressure of 1.04 $kg.cm^{-1}$. The crops were incubated in the dark at a temperature of 25 ± 2°C **(Srichuay *et al*. 2018; Hamza &Ali 2017)**.

A sensitive electronic balance was used to determine the wet and dry weights of the callus and the vegetative growths resulting from the re-differentiation stage and the roots resulting from the rooting process. The growths were extracted from the bottles they were grown in and placed on filter paper. The remaining nutrient medium was removed using a surgical blade and the wet weight of the growths was calculated, then the growths were dried. In an electric oven at a temperature of 70°C until the weight is stable, the dry weight of the growths was calculated for the entire experiment (**Al-Dabagh & Salih 2020)**.

The experiments were carried out in a CRD design, with factorial experiments, and the results were analyzed using the Genestate statistical program, with ten replications and at a probability level of 5%.

RESULTS AND DISSCUSSION

Effect of 2,4-D concentrations on the average wet weight of callus

Effect of 2,4-D concentrations on the wet weight of callus. The results in Table (1) show that there are significant differences between the barley varieties, as the variety Bouhoth244 was significantly superior in the average wet weight of callus, as it gave an average of 156.45 mg, compared to the two varieties Ebaa99 and Ebaa265, which gave a lower average wet weight of 105.45 and 95.75 mg, respectively. The variation between varieties is due to genetic differences among them and to the difference in the content of internal hormones and their role in various growth processes, and these results were confirmed by the findings of **(Al-Jubori *et al*., 2023)**.

As for the effect of 2,4-D concentrations on the average wet weight of callus, the results in Table (1) indicate that there are significant differences between the 2,4-D concentrations, as it was observed that there was a gradual increase in the average wet weight of callus with increasing concentration of 2,4-D. The highest average wet weight of callus was achieved at the concentration of 3.0 $mg.L^{-1}$, reaching 175.70 mg. The reason for the superiority of the 2,4-D treatment in the average wet weight of callus may be due to the stimulating action of 2,4-D in urging cells to divide and increase Growth and stimulating the elongation of plant cells through its effective role in stimulating the plasticity of the cell wall, as it causes the bonds of that wall to be broken and returned to new locations under the influence of turgor pressure, which contributes to increasing the size and breadth of the cell,

so in addition to its effect on the enzymes responsible for building the components of the cell wall. And its decomposition, thus affecting the mechanical properties of that wall, most notably increasing its permeability **(Baday, 2019)** , these results were confirmed by the findings of **(Ayudhaya *et al.*, 2023)**, who indicated that 2,4-D is the most stimulating factor for callus formation when the concentration does not exceed 3.0 mg.L^{-1}, while the average wet weight of callus decreased to (157.13) mg when the concentration increased. 2,4-D to (4.5) mg. L^{-1}, the decrease in the average wet weight of callus at high concentrations of auxins may be due to the fact that high concentrations of 2,4-D may lead to inhibition of cell growth and may stop them from dividing and expanding **(Toma,2022)**. The lowest average wet weight of callus was recorded. In the comparison treatment, it was 41.83 mg.

It is noted from the same table that the interaction between the varieties and the concentrations of 2,4-D indicates the presence of significant differences, as the lowest average wet weight of callus was recorded in the variety Ebaa 265, amounting to 32.00 mg in the comparison treatment, while the highest average wet weight of callus was achieved in the variety Bouhoth244, amounting to 225.70. mg at a concentration of 3.0 mg.L^{-1} of 2,4-D, this may be due to the fact that auxins are important hormones with high effectiveness in plant tissue cultures, as they help cell division and callus formation **(Fadladeen & Toma,2022)**.

Table (1): Effect of 2,4-D concentration (mg.L^{-1}) on the average wet weight (mg) of callus of barley varieties after four weeks of planting.

Concentration 2,4-D mg.L^{-1}	**Varieties**			**Average 2,4-D**
	Ebaa265	Ebaa99	Bouhoth244	
0	32.00	37.00	56.50	41.83
1.5	86.20	94.20	126.20	102.20
3	145.80	155.60	225.70	175.70
4.5	119.00	135.00	217.40	157.13
LSD5%	1.23**			0.71**
Varieties average	95.75	105.45	156.45	
LSD5%	0.62**			

The results in Table (2) show that there are significant differences between the barley varieties, as the variety Bouhoth244 was significantly superior in the average dry weight of callus, as it gave an average of 46.55 mg, compared to the two varieties Ebaa99 and Ebaa265, which gave a lower average dry weight of 31.51 and 28.01 mg, respectively. The discrepancy is due to among the varieties, it is attributed to the genetic differences among them and to the difference in the content of internal hormones and their role in various growth processes, and these results were confirmed by the findings **(Xu *et al.*, 2022)**.

As for the effect of 2,4-D concentrations on the average dry weight of callus, the results in Table (2) indicate that there are significant differences between the 2,4-D

concentrations, as it was observed that there was a gradual increase in the average dry weight of callus with increasing 2,4-D concentration. The highest average dry weight of callus was achieved at the concentration of 3.0 mg.L^{-1}, reaching 52.76 mg. The reason for the superiority of 2,4-D treatments in the average dry weight of callus may be due to the stimulating action of 2,4-D in urging cells to divide and increase Growth, as the growth regulator 2,4-D affects plant metabolism by manufacturing proteins, thus influencing the effectiveness of enzymes, respiration, and cell division **(Nasrallah *et al*., 2015)**, while the average dry weight of callus decreased to 46.11 mg when the concentration of 2,4-D increased. to (4.5) mg. L^{-1}, the decrease in the average dry weight of callus at high concentrations of auxins may be due to the fact that high concentrations of 2,4-D may lead to inhibition of cell growth and perhaps stop them from dividing and expanding these results confirm what It was found by **(Al-Jubori *et al*.,2023)** and the lowest average dry weight of callus in the comparison treatment was 12.05 mg.

It is noted from the same table that the interaction between varieties and 2,4-D concentrations indicates the presence of significant differences, as the lowest average dry weight of callus was recorded in the Ebaa265 variety, amounting to 8.10 mg in the comparison treatment, while the highest average dry weight of callus was achieved in the Bouhoth244 variety, amounting to 68.38 mg at concentration 3.0 mg.L^{-1} of 2,4-D.

Table (2) Effect of 2,4-D concentration (mg.L^{-1}) on the average dry weight (mg) of calluses of barley varieties four weeks after planting.

Table (2): Effect of 2,4-D concentrations on the average dry weight of callus.

Concentration 2,4-D mg.L^{-1}	**Varieties**			**Average 2,4 -D**
	Ebaa265	Ebaa99	Bouhoth244	
0	8.10	12.18	15.86	12.05
1.5	25.68	28.16	37.66	30.50
3	42.90	47.00	68.38	52.76
4.5	35.34	38.68	64.30	46.11
LSD5%	0.81**			0.47**
Varieties average	28.01	31.51	46.55	
LSD5%	0.41**			

Effect of BA concentrations on the average of number of differentiated vegetative growths of callus treated with Harmel seeds extract

The results in Table (3) show that there are significant differences between the barley varieties, as the variety Bohoth244 was significantly superior in the average number of differentiated vegetative growths, as it gave an average of 6,82 shoots callus, compared to the two varieties Ebaa99 and Ebaa265, which gave a lower average number of differentiated vegetative growths of 4,86 and 3,07 branches.callus^{-1} respectively, may be due to genetic differences among them, as well as the difference in their content of internal growth

regulators, which may be attributed to genetic factors that control the process of building hormones and their accumulated quantities within the plant and their role in growth depending on the type of growth regulator **(Hairuddin *et al*., 2023; Seleem & Taha, 2021).**

As for the effect of BA concentrations on the average of the number of differentiated vegetative growths, the results in Table (3) indicate that there are significant differences between the concentrations of BA, as it was observed that there was a gradual increase in the average of the number of differentiated vegetative growths with increasing BA concentration, as the highest average of the number of differentiated vegetative growths was achieved when The concentration of 1.0 mg.L^{-1} reached 6,86 shoots.callus^{-1}. The reason for the superiority of BA treatment in the average number of differentiated vegetative growths may be due to the stimulating action of BA in urging cells to divide and increase growth. This is explained by the fact that the presence of cytokines is necessary in the process of organic formation and improving the quality of formed growths **(Asami & Hitoshi, 2015)**, these results were confirmed by the findings of **(Hairuddin *et al*., 2023)** who indicated that BA is the most stimulating for the re-differentiation of growths when the concentration does not exceed 1.0 mg.L^{-1}, while the average number of differentiated vegetative growths decreased to (4,52) branches.callus^{-1} When the concentration of BA is increased to (3.0) mg.L^{-1}, the decrease in the average of the number of differentiated vegetative growths at high concentrations of cytokinins may be attributed to the fact that high concentrations of BA may lead to inhibition of cell growth and may stop them from dividing and expanding these results confirm what was found by **(Toma, 2022; Al-Obaidy & Khierallah, 2017),** and the lowest average number of differentiated vegetative growths was recorded in the comparison treatment, amounting to 2,91 shoots.callus^{-1}, it is noted from the same table that the interaction between the varieties and BA concentrations indicates that there are no significant differences.

It is noted from the same table that the interaction between varieties and BA concentrations indicates the presence of no significant differences **(Mohamad *et al*., 2022;Marquez *et al*., 2019).**

Table (3): Effect of BA concentration (mg.L^{-1}) on the average number of differentiated vegetative growths (shoot.callus^{-1}) from seed calluses of barley varieties treated with Harmel seeds extract four weeks after planting.

Concentration BA mg.L^{-1}	Varieties			Average BA
	Ebaa265	Ebaa99	Bouhoth244	
0	1.43	2.71	4.57	2.91
1	5.14	6.71	8.71	6.86
2	3.29	5.29	7.57	5.38
3	2.43	4.71	6.43	4.52
LSD5%	Ns			0.32**
Varieties average	3.07	4.86	6.82	
LSD5%	0.28**			

Figure (1): The effect of concentrations of 1 mg.L^{-1} of BA on the average of the number of differentiated vegetative growths from callus varity Bouhoth244.

Effect of BA concentrations on the average length of differentiated vegetative shoots of callus treated with Harmel seeds extract

The results in Table (4) show that there are significant differences between the barley varieties, as the variety Bouhoth244 was significantly superior in the average length of vegetative growths, as it gave an average of 11.68 cm compared to the two varieties Ebaa99 and Ebaa265, which gave a lower average length of vegetative growths of 9.22 and 7.03 cm, respectively. The variation between varieties is due to genetic differences among them and to the difference in the content of internal hormones and their role in various growth processes, and these results were confirmed by the findings of **(Al-Dabagh & Salih, 2020).**

As for the effect of BA concentrations on the average length of vegetative growths, the results in Table (4) indicate that there are significant differences between BA concentrations, as the highest average vegetative growth length was achieved in the comparison treatment, reaching 13.13 cm. The reason may be due to the superiority of the comparison treatment in the average growth length. The vegetative growth is due to the stimulating effect of cytokinins in cell division and increased growth, while the average length of the vegetative growths decreased to 2.67 cm when the concentration of BA increased to (3.0) mg.L^{-1}. The decrease in the length of the differentiated vegetative growths at high concentrations of cytokinins may be attributed to the fact that the concentrations High levels of BA may inhibit cell growth.

It is noted from the same table that the interaction between varieties and BA concentrations indicates the presence of significant differences, as the lowest average length of vegetative growths was recorded in the Ebaa265 variety, amounting to 1.24 cm at the concentration of

3.0 mg.L^{-1}, while the highest average length of vegetative growths was achieved in the Bouhoth244 variety, amounting to 16.59. cm when comparing treatment (**Hussain *et al*.,2021; Jones & Ljung, 2011).**

Table (4): Effect of BA concentration (mg.L^{-1}) on the average length of differentiated vegetative shoots (cm) from callus produced from seeds of barley varieties treated with Harmel seeds extract after four weeks of planting.

Concentration BA mg.L^{-1}	Varieties			Average BA
	Ebaa265	Ebaa99	Bouhoth244	
0	10.51	12.30	16.59	13.13
1	8.29	11.66	13.60	11.18
2	8.09	10.46	12.19	10.24
3	1.24	2.46	4.36	2.69
LSD5%	0.25**			0.15**
Varieties average	7.03	9.22	11.68	
LSD5%	0.13**			

Effect of IBA concentrations on the root number of vegetative growths treated with Harmel seeds extract

The results in Table (5) show that there are significant differences between the barley varieties, as the variety Bouhoth244 was significantly superior in the average number of roots, as it gave an average of 14,75 in Bouhoth244 compared to the two varieties Ibaa99 and Ibaa265, which gave a lower average number of roots, amounting to 13,25and 11,11 Sequentially, the variation between varieties is due to genetic differences among them and to the difference in the content of internal hormones and their role in various growth processes **(Fadladeen & Toma , 2022 ; Al-Marsumy & Jarallah, 2019).**

As for the effect of IBA concentrations on the root number average, the results in Table (5) indicate that there are significant differences between the IBA concentrations, as it was observed that there was a gradual increase in the root number average with increasing IBA concentration, as the highest root number average was achieved at the concentration of 2.0 mg.L^{-1}. It reached 18,29 Bouhoth244, the reason for the superiority of IBA treatment in the number of roots may be due to the stimulating action of IBA in urging cells to divide and increase growth, as auxins work on the success of root formation using high concentrations of IBA. This is explained by the fact that auxin, in addition to its role in stimulating apical dominance at high concentrations certainly, it also has a role in the development and specialization of cells to form roots when used at other concentrations **(Al-Jubori *et al*.,2023)** , these results were confirmed by what the researchers reached **(Toma,2022)**, while the average number of roots decreased to 13,95 root.plant^{-1} when the concentration of IBA was increased. To 3.0 mg.L^{-1}, the decrease in the number of roots at high concentrations of auxins

may be due to the fact that high concentrations of IBA may lead to inhibition of cell growth. These results confirmed what was found **(Al-taei & Al-Shamarey, 2019).** The lowest average number of roots was recorded in the comparison treatment, amounting to 6,38 Bouhoth244. The lack of root formation is explained by using low concentrations of IBA, perhaps because the optimal concentration of auxins is not reached, which is compatible with its internal level in the vegetative branches **(Sascham *et al*., 2020 ; Fahmy & Gendy, 2018)**.

It is noted from the same table that the interaction between varieties and IBA concentrations indicates the presence of significant differences, as the lowest average number of roots was recorded in the Ebaa265 variety, amounting to 4,00 root.plant^{-1} in the comparison treatment, while the highest average number of roots was achieved in the Bouhoth244 variety, amounting to 19,14 root.plant^{-1} at a concentration of 2.0 mg.L^{-1} of IBA, this may be due to the fact that auxins are important hormones with high effectiveness in plant tissue cultures, as they help in cell division and root formation **(Al-Jubori *et al*.,2023 ; Rivas *et al*.,2022)**.

Table (5): Effect of IBA concentration (mg.L^{-1}) on the average number of roots (root. plant^{-1}) resulting from seeds of barley varieties treated with Harmel seeds extract after four weeks of planting.

Concentration IBA mg.L^{-1}	**Varieties**			**Average IBA**
	Ebaa265	**Ebaa99**	**Bouhoth244**	
0	4.00	7.00	8.14	6.38
1	10.71	13.43	16.43	13.52
2	17.43	18.29	19.14	18.29
3	12.29	14.29	15.29	13.95
LSD5%	0.77**			0.45**
Varieties average	11.11	13.25	14.75	
LSD5%	0.39**			

Figure (2): The effect of the concentration of 3 mg.L^{-1} of IBA on the average number of roots formed for the varity Bouhoth244.

Effect of IBA concentrations on root length average of vegetative growths treated with Harmel seeds extract

The results in Table (6) show that there are significant differences between the barley varieties, as the variety Bouhoth244 was significantly superior in average root length, giving an average of 5.71 cm compared to the two varieties Ebaa99 and Ebaa265, which gave a lower average root length of 4.49 and 2.83 cm, respectively. The difference is due to The varieties are attributed to the genetic differences among them and to the difference in the content of internal hormones and their role in various growth processes, and these results were confirmed by the findings of **(Salman *et al.*,2022; Balzan *et al.*,2014).**

As for the effect of IBA concentrations on the root length average, the results in Table (6) indicate that there are significant differences between the IBA concentrations, as it was observed that there was a gradual increase in the root length average with increasing IBA concentration, as the highest root length average was achieved at the concentration of 2.0 $mg.L^{-1}$ It reached 6.49 cm **(Ortiz-Castro *et al.*,2020)**. The reason for the superiority of the IBA treatment in the average of root length may be due to the stimulating action of IBA in urging cells to divide and increase growth, as auxins increase growth and cell division **(Baday, 2019)**. The lowest average of root length was recorded in the comparison treatment, which was 1.64 cm.

It is noted from the same table that the interaction between varieties and IBA concentrations indicates the presence of significant differences, as the lowest average root length was recorded in the Ebaa265 variety, amounting to 0.87 cm in the comparison treatment, while the highest average root length was achieved in the Bouhoth244 variety, amounting to 8.43 cm, at the concentration of 2.0 $mg.L^{-1}$ from IBA, that this may be due to the fact that auxins are important hormones that are highly effective in plant tissue cultures, as they help in cell division **(Baday, 2019; Michniewicz *et al.*,2019; Li *et al.*,2018).**

Table (6): Effect of IBA concentration ($mg.L^{-1}$) on average root length (cm) resulting from seeds of barley varieties treated with Harmel seeds extract after four weeks of planting.

Concentration IBA $mg.L^{-1}$	Varieties			Average IBA
	Ebaa265	Ebaa99	Bouhoth244	
0	0.87	1.23	2.82	1.64
1	2.94	4.54	5.36	4.28
2	4.26	6.77	8.43	6.49
3	3.23	5.41	6.24	4.96
LSD5%	0.10**			0.06**
Varieties average	2.83	4.49	5.71	
LSD5%	0.05**			

Acclimatization

The seedlings resulting from the rooting process were acclimatized for each of the three varieties, and the roots of the seedlings were placed in a concentration of 2.5% of the fungicide Benlate for ten seconds to reduce the risk of infection by pathogens. Then the root system of the rooted seedlings was immersed in a solution containing a quarter strength of MS salts for seven days to harden them. Plants, then the rooted plants were transferred to the growing medium consisting of (Zamij and peat moss). After that, the rooted plants were planted in anvils and watered with distilled water. Plastic covers were placed to maintain the high level of humidity, and care was taken to gradually remove the covers from them.Observations were taken about the percentage of the success of acclimated plants for all varieties after two weeks of planting reached 87%.

CONCLUSION

The Plant Growth Regulators have it is of great importance in growing plant tissue, as auxins work to encourage the induction of callus and roots, and cytokinins work to regeneration vegetable growth in the varieties of barely used (Bouhoth244, Ebaa99 and Ebaa265).

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