Propagation of Chia Plants Using Plant Tissue Culture Technique

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Abstract

Back Ground: The plant tissue culture technique is one of the biotechnologies that means isolating a plant cell, tissue or organ, sterilizing it and cultivating it on sterile artificial food media under conditions completely free of pathogens, and then the transplanted part develops into a complete plant similar to the original from which it was taken. Under controlled environmental conditions of temperature, humidity and lighting, as defined by others as sterile cultivation of cells, tissues, organs and their components under certain chemical and physical conditions in vitro.

Objectives: This study aimed to propagate the medicinal chia plant using the technique of plant tissue culture.

Materials and Methods: The research was carried out in the Plant Tissue Culture Laboratory of the College of Agriculture, Anbar University. Sterilized chia seeds were sown on the MS nutrient medium equipped with the GA3 growth regulator for the purpose of obtaining vegetative growths in the cultivation tubes, at the rate of one seed per vial, using tweezers previously sterilized with alcohol and flame. After obtaining the required growths, the stem nodes were cut into pieces 1 cm long and planted on MS medium prepared with different concentrations of BA (0.5, 1, 2, 3) mg L^{-1}. And by interfering with NAA (0.1, 0.2, 0.3) mg L^{-1} with the aim of the emergence and multiplication of vegetative branches within a factorial experiment with two factors (4 × 4) with 10 replications for each treatment. 16 hours and 8 hours dark. In the rooting experiment, different concentrations of IBA (0, 1, 2) mg L^{-1} were used, interfering with BR (0, 0.1, 0.2) mg L^{-1}, and measurements were taken after one month.

Results: It was observed through the obtained results that there were significant differences between the different concentrations of growth regulators used in the experiment in the multiplication and rooting of the branches.

Conclusions: Regarding the doubling stage, there is an increase in the number of branches as the concentration of the growth regulator BA increases. For rooting, the growth regulator brassinosteroids outperformed auxin indole butyric acid in root lengths and numbers.

Keywords: Chia Plants, Gibberellin hormone, Leaves, Stem Nodes, Tissue Culture Technique

Introduction

The chia plant (*Salvia hispanica*) is one of the plants belonging to the Lamiaceae family which is under the genus of Salvia and the chia plant contains more than 900 species (1) native to Mexico and northern Guatemala. Furthermore, chia seeds contain a high oil content (40%) with 60% omega-3 fatty acids, dietary fiber (18-30%), carbohydrates (26-41%), amounts of vitamins and minerals (2), and proteins (15-24%) (3). The chia plant is characterized as of great nutritional importance (4), as its seeds represent the highest natural source of omega-6 and omega-3 which are important fatty acids in human nutrition because they reduce the risk of cardiovascular disease. In addition, it is a natural source of gluten-free protein and a high content of antioxidants that protect the seeds from microbial and chemical decomposition (5). The chia plant is among the important medicinal plants that have been used in the treatment of many diseases, as a treatment to lower sugar and blood pressure, anticancer, anti-ulcer, and anti-inflammatory (6). Because of the importance of this plant in the treatment of many diseases, especially its multiple uses interest in cultivating this plant has increased, especially in the countries where it is prevalent. Thus due to the low rate of germination of its seeds for cultivation in Iraq the technology of plant tissue culture was employed in the propagation of this plant. Where this technique is one of the important technologies that play an effective role in serving humans, especially in the field of propagating many economically important plants due to the advantages of this technique the most important of which is obtaining huge numbers of plants similar to the mother plant. In addition, it is free from pathogens and in a short time, as well as the possibility of propagation throughout the year compared to traditional cultivation (7)(8). Growth regulators play a major role in the success of plant tissue culture. It was found that the balance between auxins and cytokinins plays a major role in the emergence and multiplication of vegetative shoots of the plant, as it requires adding them in specific concentrations depending on the genetic composition of the plant, the type of plant part, its internal content of hormones and the stage of growth (9). Furthermore, cytokinins have a role in plant cell division, differentiation, and axillary bud growth (10). The presence of auxins in the nutrient medium, where auxins in low concentrations may stimulate the division and elongation of plant cells or produce the roots for the vegetative branches. Therefore, the current study aimed to study the effect of hormones on the propagation of the chia plant in vitro.

Materials and Methods

The research was carried out in the Plant Tissue Culture Laboratory of the College of Agriculture at Anbar University for the period from March 2021 to January 2022 with the aim of studying the effect of hormones on the reproduction of chia plants in vitro.

Sterilization and cultivation of seeds

The process of sterilizing the chia plant seeds was carried out by washing them with soap and water then washed with sterile distilled water, and the seeds were soaked in a sterile solution (commercial minor) with a concentration of 2% (active substance of Sodium hypochlorite (NaClO)) for 10 minutes with continuous stirring. Then the seeds were washed with sterile water three times to get rid of the harmful effect of the sterile substance. After completing the sterilization process the seeds were sown on MS medium (11) equipped with the Gibberellic acid (GA3) growth regulator for the purpose of obtaining vegetative growths in the culture tubes and at a seed per vial using tweezers previously sterilized with alcohol and flame. It is worth noting that the sterilization and cultivation process took place inside the laminar air flow hood.

Stage of emergence and propagation

The stem nodes were cut into 1 cm pieces and cultured on MS media equipped with different concentrations of BA (0.5, 1, 2, 3) mg/ L and in interaction with NAA (0.1, 0.2, 0.3) mg/ L in order for the emergence and propagation vegetative branches within an experiment factorial by two factors (4 × 4) and with 10 replications for each treatment. The cultures were incubated in the growth room at a temperature of 25±2 °C, 16 hours of illumination, and 8 hours of darkness. Measurements were taken after 30 days of culturing for the following characteristics:-

1-branch length (cm).

2- number of branches (branch. plant).

3- nodes number (node. plant).

4- number of leaves (leaf. plant).

5- fresh weight (gm) and dry weight (gm).

Stage of rooting

Vegetative branches resulting from the propagation stage were excised and cultured on MS media equipped with different concentrations of IBA (0,1, 2) mg/ L which interacted with BR (0, 0.1, 0.2) mg/ L within a factorial experiment with ten replicates for each treatment. The cultures were incubated in the growth room under the same conditions as above. After that, the measurements were taken 30 days after culturing and included the number of roots (root. plant) and root length (cm).

Micropropagated

The weaning process was carried out for the resulting micropropagated plantlets through the rooting stage after their roots were washed with distilled water to remove the residual from the nutrient media and then they were immersed in planet fungicide solution for 5 minutes to protect them from fungal infections. Then it was cultured in anvils containing agricultural media consisting of a mixture of soil and house moss in a ratio of 1:1 and previously sterilized in the same method used to sterilize the food media. After culturing, the plants were incubated under the same conditions, taking into account the watering of the plants as needed. During the first two weeks, the plants were covered with transparent plastic covers to maintain moisture around the plants. At the beginning of the third week, the cover was gradually lifted until it was removed by the end of the fourth week. Then the plants were transferred to the greenhouse when they were ready to grow.

Experimental design and statistical analysis

The experiments were designed using the Complete Randomized Design (CRD) as factorial experiments with ten replications for each treatment to calculate the traits for the stage of propagation and rooting. Which were analyzed using the (Genstat) program, and the arithmetic mean was compared according to the least significant difference test L.S.D at the probability level of 0.05 (12).

Results

Results of the vegetative propagation stage

The effect of interaction between BA and NAA on branch length of chia plants

The results in Table (1) showed that there were significant differences between the studied treatments in the character of the length of the vegetative branches of the chia plant, it showed a decrease in the length of the vegetative branches with the increase in the concentration of benzyl adenine. The comparison treatment gave the highest average for the length characteristic compared to the rest of the concentrations of benzyl adenine. The results also showed in Table (1) that there was a significant difference for the growth regulator NAA in the average plant height, where the concentration of 0.3 mg L^{-1} gave the highest rate in plant height of 4.50 cm, whereas, the comparison treatment gave the lowest average in plant height, which was 3.70 cm. As for the interaction effect between the growth regulators BA and NAA, the results of the table show that the treatment with a concentration of 0.0 of BA with a concentration of 0.1 mg L^{-1} of NAA auxin gave it the highest rate of plant height of 9.45 cm, followed by the two treatments of 0.0 concentration of BA with 0.3 mg L^{-1} and 0.2 mg L^{-1} of NAA, which gave 6.70 and 6.30 cm, respectively. while the treatment with a concentration of 1.0 mg L^{-1} of BA with 0.1 mg L^{-1} of NAA auxin recorded the lowest plant height of 1.73 cm.

Table (1) Effect of different concentrations of BA and NAA and the interaction between them on branch length (cm) of Chia plants after four weeks of planting.

Concentration BA (mgl^{-1})	Concentration NAA (mgl^{-1})				Rate of BA
	0.0	0.1	0.2	0.3	
0.0	5.21	9.45	6.30	6.70	6.91
0.5	3.64	4.02	2.83	5.20	3.92
1	3.65	1.73	2.15	5.79	3.33
2	2.65	3.10	3.06	2.62	2.86

3	3.35	3.60	2.80	2.20	2.99
Rate of NAA	3.70	4.38	3.43	4.50	
L.S.D 0.05	BA	NAA		BA×NAA	
	0.236	0.211		0.472	

The effect of the interaction between BA and NAA on the number of branches of the Chia plant

It is clear from the results of Table (2) that there is a significant difference when adding the growth regulator BA with different concentrations in the agricultural medium for the characteristics of the number of branches of the shea plant (Figure 1) whereas, the concentration of 3.0 mg L^{-1} gave the highest average number of branches, which was 5.02 branches. $plant^{-1}$, whereas, the comparison treatment recorded the lowest rate in the number of branches, which amounted to 2.62 branches. $plant^{-1}$. As for the effect of auxin NAA, the results showed in the same table that there were no significant differences between the studied concentrations in the character of the number of vegetative branches, and this is due to the action of auxin in cell division and an increase in the length of vegetative branches not in its preparation, unlike the action of cytokinin, which works to increase the number of branches by eliminating apical dominance. The same table also shows that there are significant differences in the interaction between the growth regulators BA and NAA in the average number of branches, whereas, treatment with a concentration of 2.0 mg L^{-1} of BA with 0.0 mg L^{-1} of NAA gave the highest rate in the number of branches that reached 7.42 branches. $Plant^{-1}$, which did not differ significantly from treatment with a concentration of 3.0 mg L^{-1} of BA with 0.2 mg L^{-1} of NAA, gave an average number of branches of 6.18. $plant^{-1}$. whereas, the interaction between the BA-free treatment and the concentration of 0.1 mg L^{-1} of NAA gave the lowest rate in the number of branches, which was 2.30 branches. $plant^{-1}$.

Table (2) Effect of different concentrations of BA and NAA and the interaction between them on the number of shoots (Branch. $Plant^{-1}$) of shea plant after four weeks of planting.

Concentration BA (mgl^{-1})	**Concentration NAA (mgl^{-1})**				**Rate of BA**
	0.0	**0.1**	**0.2**	**0.3**	
0.0	2.76	2.30	2.74	2.70	2.62
0.5	4.42	4.78	5.50	4.14	4.71
1	5.30	4.30	3.90	2.90	4.10
2	7.42	4.30	4.10	2.46	4.57
3	3.00	5.50	6.18	5.40	5.02
Rate of NAA	4.58	4.24	4.48	3.52	

L.S.D 0.05	BA	NAA	BA×NAA
	0.766	0.685	1.531

The effect of the interaction between BA and NAA on the number of knots of chia plants.

The results of Table (3) showed the superiority of the treatment of benzyl adenine BA with a concentration of 0.5 mg L^{-1} by giving it the highest rate in the number of knots characteristic which reached (8.21) knots. $plant^{-1}$ did not differ significantly from the treatment with a concentration of 3.0 mg L^{-1}, which gave a knot number of 7.77 knots. $plant^{-1}$. The two treatments (1.0 and 2.0) were followed by (1.0 and 2.0) mg L^{-1}, giving them an average knot number of 6.45 knots. $plant^{-1}$, while the comparison treatment gave the lowest rate in the number of nodes, which was 5.15 knots. $plant^{-1}$. The results of the same table showed that there was no significant effect of the different concentrations of the growth regulator NAA on the average number of knots of Chia plants. As for the interaction between the growth regulators BA and NAA, the results of the table show significant differences, as the concentration of 3.0 mg L^{-1} of BA with 0.2 mg L^{-1} of NAA gave the highest rate in the number of knots reaching 10.40 knots. $plant^{-1}$, whereas, the BA-free treatment with interaction with concentration 0.2 mg L^{-1} of NAA gave the lowest rate in the number of knots amounting to 4.80 knots. $plant^{-1}$.

Table (3) Effect of different concentrations of BA and NAA and the interaction between them on the number of nodes (node. $plant^{-1}$) of Chia plants after four weeks of planting.

Concentration BA (mgl^{-1})	Concentration NAA (mgl^{-1})				Rate of BA
	0.0	0.1	0.2	0.3	
0.0	5.20	5.00	4.80	5.60	5.15
0.5	7.60	8.92	9.32	7.00	8.21
1	7.80	5.40	5.60	7.00	6.45
2	7.60	5.40	6.20	6.60	6.45
3	4.90	9.60	10.40	6.20	7.77
Rate of NAA	6.62	6.86	7.26	6.48	
L.S.D 0.05	**BA**	**NAA**		**BA×NAA**	
	0.971	0.869		1.943	

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Effect of BA and NAA on leaf count of Chia plant.

The results of the statistical analysis shown in Table (4) showed that there were significant differences when adding different concentrations of Benzyl Adenine BA (3.0, 2.0, 1.0, 0.5) mg L^{-1} in the number of leaves. The treatment with a concentration of 0.5 mg L^{-1} significantly outperformed it by giving it the highest average number of leaves that reached 13.75 leaves. $plant^{-1}$, which differed significantly from the rest of the treatments, while the comparison treatment recorded the lowest value for the average number of leaves reaching 10.75 leaves. $plant^{-1}$ and the same table indicate that there is a significant difference when adding concentrations of the growth regulator NAA to the nutritional medium according to the studied feature. The treatment with a concentration of 0.2 mg L^{-1} significantly outperformed all other treatments, while the comparison treatment recorded the lowest value for the studied trait, which amounted to 10.68 leaves. $plant^{-1}$, while the results of the interaction between BA and NAA showed a significant difference, the treatment with a concentration of 0.5 mg L^{-1} of BA with a concentration of 0.2 mg L^{-1} of NAA recorded the highest value for the average length of vegetative branches amounted to 19.40 leaves. plant-1. It was followed by treatment with a concentration of 3.0 mg L^{-1} of BA and a concentration of 0.2 mg L^{-1} of NAA, where they recorded an average number of leaves of 15.60 leaves. $plant^{-1}$. While the treatment with a concentration of 3.0 mg l^{-1} and the NAA-free treatment recorded the lowest average of 9.80 leaves. $plant^{-1}$.

Table (4) The effect of different concentrations of BA and NAA and the interaction between them on the average number of leaves (leaf. $plant^{-1}$) of Chia plants after four weeks of planting.

Concentration BA (mgl^{-1})	**Concentration NAA (mgl^{-1})**				**Rate of BA**
	0.0	**0.1**	**0.2**	**0.3**	
0.0	10.40	10.40	10.80	11.40	10.75
0.5	10.80	12.40	19.40	12.40	13.75
1	10.20	11.80	11.60	12.00	11.40
2	12.20	14.60	12.40	11.60	12.70
3	9.80	14.00	15.60	12.80	13.05
Rate of NAA	10.68	12.64	13.96	12.04	
L.S.D. 0.05	**BA**		**NAA**		**BA × NAA**
	1.517		1.357		3.034

The effect of the interaction between BA and NAA on the fresh weight of Chia plants

The results of Table (5) indicated that there was a significant difference between the concentrations of the growth regulator BA in the fresh weight of the Chia plant, where the concentration of 3.0 mg L^{-1} gave the highest average in the fresh weight of 0.4331 g, followed by the comparison treatment, which gave 0.4024 g. While the concentration of 1.0 mg L^{-1} recorded the lowest average in the fresh weight of 0.3010 g. The results also showed that the addition of the growth regulator NAA in the food medium gave significant differences between the treatments in the characteristic of the fresh weight of the shea plant, as the concentration exceeded 0.1 mg l^{-1} giving it the highest average fresh weight of 0.4266 gm. The concentration was followed by 0.3 mg L^{-1} giving it a fresh weight of 0.3862 g while the comparison treatment recorded the lowest average in fresh weight, which was 0.3297 g. As for the interaction between growth regulators BA and NAA, the concentration of 2.0 mg L^{-1} of BA in interaction with a concentration of 0.1 mg L^{-1} of NAA gave the highest fresh weight of 0.5964 g. While the interaction between the concentration of 1.0 mg L^{-1} of BA with the NAA-free treatment gave the lowest average fresh weight of 0.2404 g.

Table (5) The effect of different concentrations of BA and NAA and the interaction between them on the fresh weight (g) of chia plants after four weeks of planting.

Concentration BA (mgl^{-1})	Concentration NAA (mgl^{-1})				Rate of BA
	0.0	0.1	0.2	0.3	
0.0	0.4739	0.4559	0.3886	0.2913	0.4024
0.5	0.3136	0.3292	0.2948	0.4618	0.3499
1	0.2398	0.2441	0.2865	0.4336	0.3010
2	0.3333	0.5964	0.4249	0.2404	0.3988
3	0.2880	0.5076	0.4328	0.5041	0.4331
Rate of NAA	0.3297	0.4266	0.3655	0.3862	
L.S.D 0.05	**BA**	**NAA**		**BA×NAA**	
	0.02219	0.01985		0.04437	

The effect of the interaction between BA and NAA on the dry weight of Chia plants

The results of Table (6) indicate that there is a significant difference between the concentrations of the growth regulator BA in the average dry weight of the shea plant, where the comparison treatment recorded the highest average dry weight of 0.04237 g while the concentration of 1.0 mg l^{-1} of benzyl adenine showed the lowest dry weight of 0.0239 gm. The same table shows a significant effect of the NAA regulator on the average dry weight of chia plants, where the concentration of 0.2 mg L^{-1} gave the highest dry weight rate of 0.04294 g, while the other concentrations did not differ between them significantly. The results of the statistical analysis shown in Table (6) showed a significant effect of the interaction between the growth regulators BA and NAA, where the treatment with a concentration of 0.5 mg L^{-1} of BA and 0.2 mg L^{-1} of NAA recorded the highest dry weight rate of 0.06219 g whereas the concentration of 2.0 mg L^{-1} of BA in interaction with the NAA-free treatment recorded the lowest dry weight average of 0.02118 g.

Table (6) The effect of different concentrations of BA and NAA and the interaction between them on the average dry weight (g) of Chia plants after four weeks of planting.

Concentration BA (mgl^{-1})	Concentration NAA (mgl^{-1})				Rate of BA
	0.0	**0.1**	**0.2**	**0.3**	
0.0	0.05278	0.04288	0.03563	0.03820	0.04237
0.5	0.02329	0.02409	0.06219	0.02989	0.03486
1	0.02398	0.02031	0.02422	0.02746	0.02399
2	0.02118	0.03228	0.04284	0.02421	0.03013
3	0.02292	0.03455	0.04983	0.02301	0.03258
Rate of NAA	0.02883	0.03082	0.04294	0.02855	
L.S.D 0.05	**BA**	**NAA**		**BA×NAA**	
	0.005620	0.005027		0.011241	

Discussion

It is evident from the results of Tables (1, 2, 3, 4, 5, and 6) that there are significant differences between the different concentrations of the growth regulator BA in the studied traits, which are the length of the vegetative branches, the number of vegetative branches, the number of leaves, the number of nodes, and the fresh and dry weight. The positive effect of BA in increasing the number of branches, leaves, nodules, and fresh and dry weight may be due to the role of cytokinins in promoting vegetative growth by stimulating cell division and differentiation and attracting nutrients to the treated plant parts (13). In addition to the role of cytokinins in reducing the activity of apical dominance and the inhibitory effect of auxin in lateral buds and their role in the vascular differentiation of lateral buds, which facilitates the detection, growth, and branching of these buds (14) (15) (16). In addition, cytokinins have a role in impeding the process of protein and chlorophyll catabolism and stimulating photosynthesis enzymes, whose effect is reflected in increasing cell size and encouraging the process of division and morphological differentiation, especially when the proportion of cytokinins added to the food medium reaches a state of balance with the internal content of the plant part (17). As for the reason for obtaining the highest number of branches when treating the interaction between the concentration of cytokinin BA and auxin NAA, it may be due to the effectiveness of cytokinin in the replication events of vegetative branches increases in the presence of auxin, that is, the effect increases when they are present together in the food medium (18) (19). The presence of high concentrations of cytokinin and low auxin in the nutrient media stimulates the formation of vegetative buds that grow into branches (20). These results are in line with (21) when propagating the Damascene rose plant, where the concentration is 2.0 mg. L^{-1} of BA in combination with NAA at a concentration of 1.0 mg. L^{-1} gave the best number of vegetative branches.

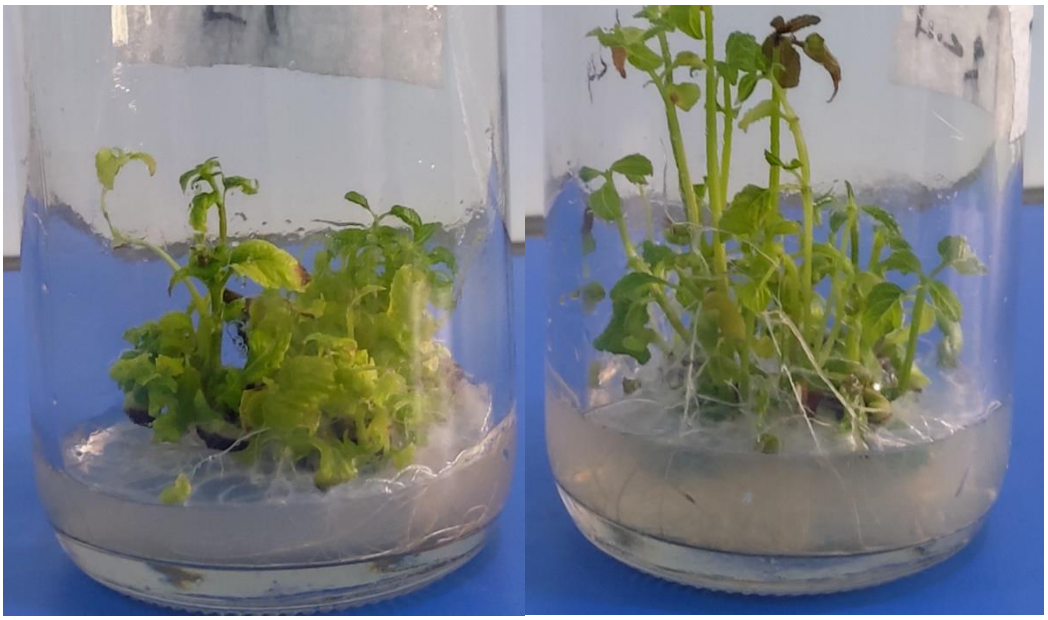

Figure (1) The effect of BA and NAA on the doubling of Chia plant branches after four weeks of cultivation on MS medium.

The effect of the interaction between IBA and BR on the average number of roots of Chia plants

Table (7) shows that there were no significant differences between the concentrations of the growth regulator indole butyric acid IBA in the characteristic of the number of roots of the Chia plant. It seems that the addition of Brassinosteroid hormone to the nutrient media was better in the formation of adventitious roots, as the concentration of 0.1 µg gave the highest average number of roots, which reached 9.47 roots. This was followed by a concentration of 0.2 micrograms, which gave an average number of roots of 8.83 roots while the comparison treatment recorded the lowest average number of roots, which was 4.44 roots. As for the interaction between the growth regulators IBA and BR, the treatment with a concentration of 0.0 mg L^{-1} of IBA and the treatment with a concentration of 0.2 µg gave the highest root number of 12.38 roots which did not differ significantly from the treatment of 0.0 mg L^{-1} of IBA with 0.1 µg of BR, which gave an average root number of 10.90. $plant^{-1}$, while the auxin and brassinosteroid-free treatment did not give any number of roots.

Table (7) Effect of different concentrations of IBA and BR and the interaction between them on the average number of roots (root.plant^{-1}) of Chia plants after four weeks of planting.

BR IBA	**0.0**	**0.1**	**0.2**	**Rate of IBA**
0.0	0.0	10.90	12.38	7.76
1	6.43	8.70	8.20	7.78
2	6.90	8.80	5.90	7.20
Rate of BR	4.44	9.47	8.83	
L.S.D 0.05	IBA	BR	IBA × BR	
	1.372	1.372	2.376	

Effect of growth regulators IBA and BR on the average root length of Chia plants.

The results of Table (8) show that there are significant differences between the concentrations of the growth regulator indole butyric acid IBA in the average root length. The treatment with a concentration of 2.0 mg L^{-1} was superior to it by giving it the highest rate of root lengths which reached 4.95 cm. This was followed by treatment with a concentration of 1.0 mg L^{-1}, which gave an average root length of 4.87 cm whereas the comparison treatment gave the lowest mean of root lengths, which was 3.64 cm. As for the effect of different concentrations of BR, the treatment with a concentration of 0.1 µg gave the highest average for the longest roots, which was 5.54 cm followed by the treatment with a concentration of 0.2 micrograms, which recorded an average of root lengths of 5.19 cm whereas the comparison treatment recorded the lowest mean of root lengths, which was 2.73 cm. The results of the statistical analysis in Table (8) also showed a significant effect of the interaction between the growth regulators IBA and BR. The treatment with a concentration of 0.0 of IBA and a concentration of 0.1 µg of BR outperformed it by giving it the highest rate of root lengths of 6.52 cm whereas, the treatment without the growth regulators, indolebutyric acid and brassinosteroids, recorded the lowest mean of root lengths, which was 0.0.

Table (8) The effect of different concentrations of BA and NAA and the interaction between them on the average root length (cm) of chia plants after four weeks of planting.

BR IBA	**0.0**	**0.1**	**0.2**	**Rate of IBA**
0.0	0.0	6.52	4.40	3.64
1.0	3.40	5.50	5.70	4.87
2.0	4.78	4.60	5.48	4.95

Rate of BR	2.73	5.54	5.19
L.S.D 0.05	IBA	BR	IBA × BR
	0.818	0.818	1.416

The results of Tables (7 and 8) show that there is a significant effect of the auxin growth regulator in stimulating the formation of roots on the branches resulting from the multiplication stage of the Chia plant (Figure 2), as the division of root primordia cells depends on the type of auxin, whether natural or added, meaning that the effects The physiology of the presence of auxins lie in the increase in cell division and it transforms specialized adult cells into meristem cells, thus forming the adventitious root meristem, whose cells divide to form roots (22). Auxins are characterized by their ability to regulate growth by stimulating elongation in plant cells. Many types of research and studies indicated that the concentration of auxin had a significant effect on rooting percentage, the number of roots and their lengths formed. The positive effect of IBA in stimulating the formation and length of roots is due to its role in encouraging the emergence of adventitious roots by stimulating cell division and elongation (23). Brassinosteroids play an important role in stimulating cell division and root elongation as a new type of plant hormone (24) (25) and in stimulating the top-down polar transfer of auxin that stimulates root growth and development (26).

These results agree with (27) when rooting stevia branches on MS medium supplemented with different concentrations of IBA and BR where the concentration gave 2.0 µg. L-1 has better rooting compared to other concentrates.

Figure (2) Effect of IBA and BA on rooting of Chia plants grown on MS medium after four weeks of cultivation.

Micropropagated stage

Four weeks after starting the micropropagation process, the plants resulting from tissue culture in the development room were transferred to grow in greenhouse conditions, and after 6 weeks of transfer to the greenhouse, the plants were successful in surviving (Figures 3 and 4) and growing by 100%.

Figure (3) Plant micropropagation

Conclusion

The addition of BA at the doubling stage stimulated an increase in the number of vegetative branches, as it achieved the highest rate at a concentration of 3.0 mg L-1, while we notice a decrease in the length characteristic, and this may indicate an inverse relationship between increasing the number and length of branches. The addition of auxin with the presence of cytokinin achieved the highest increase in the number of vegetative branches, which indicates the existence of a cooperative relationship to a certain extent. With regard to rooting, we conclude that brassinosteroids have an effective role in the rooting process compared to indole butyric acid.

References

1.Jamboonsri W., Phillips T. D., Geneve R. L., Cahill J. P., Hildebran D. F. Extending the range of an ancient crop, *Salvia hispanica* L., a new & ω-3 source. Genetic Resources and Crop Evolution, 2012; 59, p. 171-178.

2.Otondi, E. A., Nduko, J. M., and Omwamba, MPhysico-chemical properties of ex-truded cassava-chia seed instant flour. Journal of Agriculture and Food Research, 2020; 2, Article 100058.

3. Pereira, E., Encina-Zelada, C., Barros, L., Gonzales-Barron, U., Cadavez, V., and Fer-reira, I. C. F. R. Chemical and nutritional characterization of Chenopodium quinoa Willd: (quinoa) grains: A good alternative to nutritious food. Food Chemistry, 2019; 280, 110 - 114.

4. FAO/WHO. Report joint FAO/WHO Food Standards Programme. Codex Alimentarius Commission. Thirty-ninth Session. Italy. 2016; pp: 80.

5. Ullah, R, Nadeem, M., Khalique, A., Imran, M., Mehmood, S., Javid, A., and Hus - sain, J. Nutritional and therapeutic perspectives of Chia. *Saliva hispanica* L. A review. Journal of Food Science Technology, 2016; 53, 1750 - 1758.

6. Yadav, A., Joshi, A., Kothari, S. L., Kachhwaha, S., and Purohit, S. Medicinal, nutritional and industrial applications of Salvia species: A revisit. International Journal of Pharmaceutical Sciences Review and Research, 2017; 43(2), 27–37.

7. Gupta, S. and Ibarakl, Y. Plant Tissue Culture Engineering. Vol.6.The Background. Springer. 2006; 469 pages.

8. Kasumi, M., Takastu, Y. and Manab, T. Callus formation and plant regenera -tion from root explan of gladiolus (Gladiolus grandiflora Hort.).J.JPN.Soc. 2004; Vol. 4. No.1.

9. Fahmy, Fikri Jalal Muhammad. Plant tissue culture - Scientific Books House for Publishing and Distribution. Egypt - Cairo - Faculty of Agriculture - Assiut University. 2003.

10. Dello loio, R. Cytokininus determine *Arabidopsis* root meristem size by controlling cell differentiation. Curr. Biology, 2007; 17(18) : 678-682.

11. Murashige, T., and Skoog, F. A revised medium for rapid growth and bio assays with Tobacco tissue cultures. Physiologia Plantarum, 1962; 15: 473–497.

12. Al-Sahuki, Medhat and Wehaib, Kareema Ahmed. Application in design and analysis of experiments. Ministry of higher education and scientific research. Iraq. 1990.

13. Hartmann, H. T., Kester, D. E., Davies, F. T. and Geneve, R. L. Plant Propagation and Practices, sixth Edition. Hall Inc., New Jersey, USA. 1997; pp: 685.

14. Abdul-Hussein, M. A. A., AlTfeely, A.K.H. and Al-Jubouri, S. A. Effect of growth regulators and media composition the micro propagation of Nyctaginaceae Bougainvillea glabra. Kufa Journal of Agricultural Sciences. 2010; 2 (1): 100-108.

15. Gindia, H. Physiology of Fruit Trees Latest Methods in the Treating the Agricultural, Growing and Production of Fruit Trees in Various Lands. Arab House for publication and Distribution. Cairo .Egypt. 2003.

16. Hopkins, W. G. and Hiiner, N. P. A. Introduction to Plant Physiology 3rd edition. John Wiley and Sons, inc. 2004.

17. Al-Rifai, A.T. and Al-Shwbaki, S.A. Techniques of the 21 st Centuryto ImprovePlantbyTissue Culture. The Arab ThoughtHouse. Cairo. Egypt. 2002.

18. Mohamed, A. A. K and Younis, M.A. Principles of Plant physiology. Baghdad University. Ministry Of Higher Education and Scientific Research. Iraq. 1991.

19. Neumann, K. H., A. Kumar and J. Imani. Plant cell and Tissue Culture a tool in biotechnology .Springer , Berlin . Germany. 2009.

20. Skoog, F. and Miller, C.O. Chemical regulation of growth and organ formation in plant tissue cultured in vitro. Symp. Soc. Exp. Biol. 1957; 9:118-131.

21. Hamza, Musa Mohammed. Effect of auxin (NAA) and cytokinin (BA) on ex vivo micropropagation of Rosa damascene Mill. 2011.

22. Ibrahim, K . M and Abdul – Hameed, S. The Narseries . The Uni . of Musil . Univ .Press . 2001.

23. Hartmann , H.T., Kester, D. E., Davies, F.T. and Geneve, R.L. Plant propagation . Principles and Practices. 7th ed. New Jersey . 2002.

24. Al-Khafaji, M. A. Plant growth regulators their gardening applications and horticultural uses. 2014.

25. Sasaki, H. Brassinolide promotes adventitious shoot regeneration from cauliflower hypocotyl segments. Plant Cell, Tissue and Organ Culture. 2002; 71 : 111-116.

26. Hedden, Peter and Stephen, G. Thomes. Plant Hormone Signaling. Harpenden, UK. 2006.

27. Mohammed, M. A., and Almehemdi, A. F. Impact of benzyladenine, brassinolide and IBA on the proliferation of shoots and initiation of roots in stevia rebaudiana bertoni. Indian Journal of Ecology, 2021; 48(July), 335–337.

إكثار نبات الشيا باستخدام تقنية زراعة الأنسجة النباتية

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الملخص

خلفية عن الموضوع: تقنية زراعة الانسجة النباتية هي احدى التقانات الاحيائية التي تعني عزل خلية او نسيج او عضو نباتي وتعقيمه وزراعته على اوساط غذائية اصطناعية معقمة وتحت ظروف خالية تماما من المسببات المرضية ومن ثم تطور الجزء المزروع الى نبات كامل مشابه للأصل الذي أخذ منه ويتم ذلك تحت ظروف بيئية مسيطر عليها من الحرارة والرطوبة والإضاءة، كما عرفها البعض الاخر بانها الزراعة المعقمة للخلايا, الانسجة، الاعضاء ومكوناتها تحت ظروف كيميائية وفيزيائية معينة خارج الجسم الحي.

الهدف من البحث: هدفت هذه الدراسي الى اكثار نبات الشيا الطبي باستخدام تقنية زراعة الانسجة النباتية.

المواد وطرائق العمل: نفذ البحث في مختبر زراعة الانسجة النباتية التابع لكلية الزراعة جامعة الانبار. زرعت بذور نبات الشيا المعقمة على الوسط الغذائي MS المجهز بمنظم النمو GA_3 لغرض الحصول على نموات خضرية في انابيب الزراعة وبواقع بذرة لكل قنينة (Vial) باستخدام ملاقط معقمة مسبقا بالكحول واللهب. وبعد الحصول على النموات المطلوبة قطعت العقد الساقية الى اجزاء بطول 1 سم وزرعت على وسط MS مجهز بتراكيز مختلفة من BA (0.5 ، 1 ، 2 ، 3) ملغم لتر$^{-1}$ وبالتداخل مع NAA (0.1 ، 0.2 ، 0.3) ملغم لتر$^{-1}$ بهدف نشوء وتضاعف الافرع الخضرية ضمن تجربة عاملية بعاملين (4 × 4) وبواقع 10 مكررات لكل معاملة وحضنت الزروعات في غرفة النمو (Growth room) على درجة حرارة 25±2 م^{o} واضاءة 16 ساعة وظلام 8 ساعات. وفي تجربة التجذير استخدمت تراكيز مختلفة من IBA (0، 1 ، 2) ملغم لتر$^{-1}$ وبالتداخل مع BR (0، 0.1 ، 0.2) ملغم لتر$^{-1}$ واخذت القياسات بعد شهر.

النتائج: لوحظ من خلال النتائج المستحصل عليها وجود فروق معنوية بين التراكيز المختلفة لمنظمات النمو المستخدمة في التجربة في تضاعف الافرع وتجذيرها.

الاستنتاجات: فيما يخص مرحلة التضاعف هناك زيادة في عدد التفرعات كلما زاد تركيز منظم النمو BA وبالنسبة للتجذير فقد تفوق منظم النمو البراسينوستيرويدات على الاوكسين اندول بيوترك اسد في اطوال الجذور واعدادها.

الكلمات المفتاحية: نبات الشيا ، هرمون الجبرلين ، الأوراق ، العقد الساقية، تقنية زراعة الانسجة.