RESEARCH ARTICLE
Published 30th June 2025
https://doi.org/10.21070/nabatia.v13i1.1655

Induction Callus From Cotyledons of *(Atropa belladonna* L.*)* and Evolution Ionic Content Using Plant Tissue Culture

Ekhlas Meteab Ahmed[1*], Nadhim Salim Ghanim[2], Wijdan Saadi Aziz[3]

[1,2]Dept of Horticulture and landscape design ,College of Agriculture , University of Diyala ,Iraq

[3]Dept .of Biology, College of Education for pure science, Tikrit University ,Iraq

*Corresponding authors email: ekhlasmeteab@uodiyala.edu.iq

Abstract. *This study dealt with the induction of callus tissue of the belladonna plant Atropa belladonna L. through the establishment of tissue cultures by growing cotyledons on Whites-(1934) medium (WM) supplied with different concentrations of BAP (0.0, 5.0, and 1.5) mg.L-1 and 2 4-D at level (0.0, 1.5, and 2.0) mg.L-1, Callus tissue implantation at different levels of Salicylic acid, tyrosine, Jasmonic acid, PEG, and NaCl separately. The results showed that the best fresh and dry weight was obtained from the induction of callus when adding BAP At level 1.5 mg.L-1 mixed with 2,4- D At level 2.0 mg.L-1 reached 3.97 g and 0.649 mg on straight. The results showed the highest percentage of nitrogen reached 0.98 µg.gm dry weight when adding Jasmonic acid at level 75 mg.L-1, and the highest percentage of phosphorus reached 0.67 µg.gm dry weight when adding PEG at level 0.5 gm, and the highest percentage of potassium reached 0.98 µg.gm dry weight when adding Salicylic acid at a concentration of 75 mg.L-1 , and the highest increase in calcium reached 637.0 µg.gm dry weight when adding Jasmonic acid at level 75 mg.L-1 .and the highest percentage of sodium reached 185.3 µg.gm dry weight when adding tyrosine at level 40 mg.L-1, and the highest increase in chlorine reached 178.2 µg.gm dry weight when adding NaCl at level 75 mg.L-1.*

Keywords: *Atropa belladonna* L., *Ionic contents, Callus, in vitro.*

INTRODUCTION

The belladonna plant, Atropa belladonna L. It belongs to the Solanaceae family and is considered one of the most important medicinal plants. This family includes 90 genera and 2,000 species of plants. And the plant is native to Europe, in its southern regions, from there it spread to Central and Western Asia., reaching the Himalayas and to Morocco and Algeria in the south, and it is grown in England, France, and the United States of America. The belladonna plant has been known since ancient times, as the first diagnosis of the plant was in 1504 AD [1] . The Atropa genus includes a group of four species of perennial herbs. The most famous species is Atropa belladonna L. , which grows in the form of a shrub reaching a height

of 1.5 m. The root is branched in the from of a cone. The leaves are large, oval in shape, with a pointed base and the top. They are yellowish-green in color, and the flowers are small, up to Its length is 2.5-3.5 cm, and it is carried on a downward-curving flower stand. The corolla leaves purple in color, while the fruits are small, semi-spherical in shape, soft and juicy, 3-10 mm in diameter, green in color, and turn purple upon maturity [2]. Seeds It is kidney-shaped, coffee-yellowish in colour, and the seed wall is solid, which is difficult to germinate [3] . Callus is a tissue that arises when plant organs and tissues are injured. It is an undifferentiated tissue so the process of inducing callus tissue is called callus tissue induction. [4] defined callus as undifferentiated parenchyma cells that arise in areas of cuts or wounds in plant parts. He explained that the process of inducing callus on the planted plant part goes through three important stages: stimulation, division, and differentiation. Callus tissue is produced in the vegetative part of the outer cortex cells. The dividing cells generate pressure on the epidermal cells. The continuous cell division results in callus tissue on the plant part. Young tissues are the most likely to produce callus. Callus was obtained from plant parts with high cells. Specialization [5]. Among the studies conducted in this field are those conducted by [6] on the Fagonia indica plant in which they showed that adding salicylic acid to the MS nutrient medium gave a significant increase in the fresh weight of callus. [7] showed in a study on the Coleus blumei plant that adding thyrosine acid at a concentration of 0.30 g l-1 to the B5 nutrient medium gave Highest weight for fresh and dry callus, reaching 15.9 g and 3.12 g, respectively. [8] explained that growing Oryza sativa callus on MS medium supplied with different concentrations of polyethylene glycol (PEG) had an effect on the percentage of callus formation and on its fresh and dry weight, as the concentration of 1.25 g L-1 gave the highest percentage to form callus and the highest fresh and dry weight of callus also gave the best concentration of (N , P) elements in the callus tissue. The research aimed to determine the possibility of inducing callus tissue of Atropa belladonna L. using cotyledon leaves and a nutrient medium containing different concentrations of growth regulators (2,4-D and BAP). And knowing the effect of adding some stimulants, chemical agents, and abiotic stresses on the callus tissue and its content of some nutrients by growing the callus on nutrient media containing different levels of Salicylic acid, tyrosine, Jasmonic acid, PEG, and NaCl.

MATERALS AND METHODS

a. Place of conducting the experiment

The study experiments were conducted in the Tissue Culture Laboratory - College of Agriculture - Tikrit Univ. on the Belladonna plant during the period from 11/1/2021 to 9/15/2022.

b. Preparing the nutrient medium

Use the well-known nutrient medium Whites-(1934) (WM) [9] in ready-made powder from HI media Ltd. To grow plant parts and induce callus. 4.43 g of the ready-made powder was weighed into the nutrient medium, in addition to adding sucrose (30 g.l-1). All ingredients are mixed together. on the mixing device to dissolve them, then growth regulators were added according to the experiment and the volume was completed to liter with distilled water, and the pH of the medium was adjusted to 0.1±5.7 with a solution of hydrochloric acid. (HCl) or

sodium hydroxide (NaOH) solution 0.1 m. Add the agar (Agar-Agar) in an amount of 7 gm.l-1 to the medium, and place it on the hot plate magnetic stirrer and heat it below the boiling point to dissolve the agar and homogenize the nutrient medium, then pour the medium directly into glass bottles with dimensions (3 x 15 cm) by 20 ml/bottle, which was closed with its own cap, with 10 replicates for each concentration, Then it is sterilized in a pressure vessel at 121°C for 20 minutes under a pressure of 1.04 kg/cm2, then the sterile medium is removed and left to solidify at room temperature until use.

c. Sterilize the seeds and plant them in a sterile environment

The seeds of Atropa belladonna L. which was obtained from the Medicinal and Aromatic Plants Unit of the College of Agriculture - University of Baghdad, under running water for 20 minutes, then washed with regular washing powder for 7 minutes with constant stirring, then washed by placing it in a strainer under running water for 30 minutes, then transferred To the cultivation table, a 10% NaOCl solution was added to it, volume: volume, for 10 minutes, (prepared from the minor solution Commercial oil containing 0.6% sodium hypochlorite) was then washed with distilled and sterile water five times in a row for 3 minutes each time to remove the harmful effect of the sterilizing substance. After the sterilization process was completed, the seeds were transferred and placed in sterile Petri dishes containing filter paper and left five minutes to dry. Thus, they became The seeds were prepared for planting, and then planted in Whites- (1934) (WM) medium free of growth regulators.

d. Induction of callus tissue

Adding different concentrations of 2,4-D and BAP

With the aim of inducing callus from plant parts (cotyledonous leaves), after the growth of seedlings, cotyledon leaves with a length of 0.8 cm were taken in order to induce callus tissue. The plant parts were grown on Whites- (WM) (1934) medium prepared with different concentrations of 2,4-D. (0.0, 1.5 and 2.0)mg.L-1 mixed with BAP at concentrations (0.0 , 5.0 and 1.5 mg.L-1). After planting all the plant parts, at a rate of 10 plant parts for each concentration, the plants were transferred to the growth room under a lighting intensity of 1000 lux, with a daily alternation of 16 hours of light followed by 8 hours of darkness, equipped with white fluorescent tubes and at a temperature of 25 ± 1. mº, and after 5 weeks, soft and dry callus and softening of the formed callus were measured. (g) using a sensitive scale. As for the dry weight (mg), it was measured after drying the callus samples in an electric oven at a temperature of 65 °C until the weight was stable using a sensitive balance. In light of the results, The concentration of growth regulators BAP and 2,4-D(1.5 mg.l-1 BAP and 2.0 mg.L-1 2,4-D) was adopted in subsequent experiments as it gave the best results.

Adding some stimulants, chemical agents, and abiotic stresses

150 mg of callus tissue formed was taken from the treatment that gave the best results 2,4-D and BAP (1.5 mg.l-1 BAP and 2.0 mg.l-1 2,4-D) was taken and grown on Whites- (1934) medium. WM) prepared with salicylic acid at concentration(0.0 , 25, 50, 75) mg.l-1 and Tyrosine with a concentration of (0.0, 20, 40, 60) mg.l-1 and Jasmonic acid with a concentration of (0.0, 25, 50, 75) mg.l-1 and (PEG) Polyethylene Glycol which Molecular weight 6000 (g mol-1) and concentration (0.0, 0.5, 1.0 ,1.5) g.l-1, and NaCl at a concentration

of (0.0, 25, 50, 75) mg.l-1, separately, at a rate of 10 replicates for each concentration. The cultures were incubated in the growth room at a temperature of 25±1°C and intense Illumination of 1000 lux for 16 hours of light and 8 hours of darkness/day, and it was done It takes on the following characteristics five weeks after planting.

1. The fresh weight (mg) was calculated according to the method mentioned in the previous experiment.
2. The dry weight (mg) was calculated according to the method mentioned in the previous experiment.
3. The diameter of the callus (mm) was calculated using the Vernier caliper
4. Estimation Concentrations of nutrients (N, P, K, Na, Ca, Cl) µg.gm dry weight.

Nutrients in callus tissue were estimated in laboratories at the Ministry of Science and Technology.

e. Digestion of plant samples

For all the aforementioned treatments, 0.5 g of ground callus was taken separately and digested according to the method of [10] by adding 10 ml of a mixture of concentrated sulfuric acid H2SO4 and perchloric acid HCIO4 in a ratio of 1:4 and heated until the solution became clear in colour, then diluted to 50 cm3 for the purpose of ion determination.

Determination of nitrogen (*Micrograms.g-1*)

Determination of nitrogen using a micro-kjeldahl device according to the method mentioned by [11].

Determination of phosphorus (micrograms.g-1)

Phosphorus was determined by the method [12] using ascorbic acid and ammonium molybdate. The absorbance was read with a Spectrophotometer UV-VIS model D 80, at a wavelength of 662. nm, [13].

Determination of potassium, sodium, calcium and chloride ions (µg.gm-1)

Potassium, sodium and calcium ions were estimated by following the method [14] using a flame meter device. While the chloride ion was estimated using the method of plating with silver nitrate and according to the method of the same researcher above.

f. Statistical analysis

All experiments were carried out using a completely randomized design (CRD), and the statistical program Statistical Analysis System -SAS (2012) was used to analyze the data to study the effect of different treatments on the studied traits. Each treatment included ten replicates, and each replicate contained one plant part. The (Least Significant Difference-LSD) test was used to compare the means at the level of 0.05 [15].

RESULTS AND DISCUSSION

a. Effect of adding different concentrations of 2,4-D and BAP on the fresh and dry weight of Atropa belladonna L. callus.

Table (1) shows that adding growth regulators 2,4-D and BAP It had an effect on the weight of fresh callus, as the interaction treatment between 2,4-D at levels 2 mg L-1 with BAP at levels 1.5 mg L-1 gave the highest fresh weight. It reached 3.97 g , which was significantly superior to the rest of the interactions, while the interaction treatment between 2,4-D gave a concentration 1.5 mg L-1 with BAP at levels 0.0 mg L-1, the lowest fresh weight was 0.78 g, while the comparison treatment did not give any callus. As for the dry weight, adding regulators was a significant effect on it, as the interaction treatment between 2,4-D gave at levels of 2 mg L-1 with BAP at levels 1.5 mg L-1, the highest dry weight reached 0.649 mg, which exceeded Significantly compared to the rest of the interactions, while the interaction treatment between 2,4-D at a concentration of 1.5 mg L-1 with BAP at levels 0.0 mg L-1 gave the lowest dry weight of 0.038 mg, while the comparison treatment did not give any callus.(1) Figure.The reason for the increase in fresh and dry weight of callus with the addition of auxins and cytokinins may be that the plant part has an increased ability to stimulate callus when the medium is prepared using regulators, which encourages cell division and growth processes by interfering with the appropriate compositions in addition to increasing the production of important and necessary contents to support division and growth such as amino acids and proteins, which leads to an increase in the biomass of callus and an increase in its dry weight rates. [16].

Table 1. Effect of adding different concentrations of 2,4-D and BAP and their interaction on the fresh and dry weight of Atropa belladonna L. callus grown on Whites- (WM) (1934) medium.

Concentration mg.L-1		fresh weight (g)	dry weight (mg)
2,4-D	BAP		
0	0.0	0.0	0.0
	0.5	0.89	0.032
	1.5	0.93	0.119
1.5	0.0	0.78	0.038
	0.5	1.69	0.117
	1.5	2.76	0.135
	0.0	0.99	0.074
2	0.5	2.93	0.327

	1.5	3.97	0 .649
L.S.D (0.05)	0.658*	0.216*	

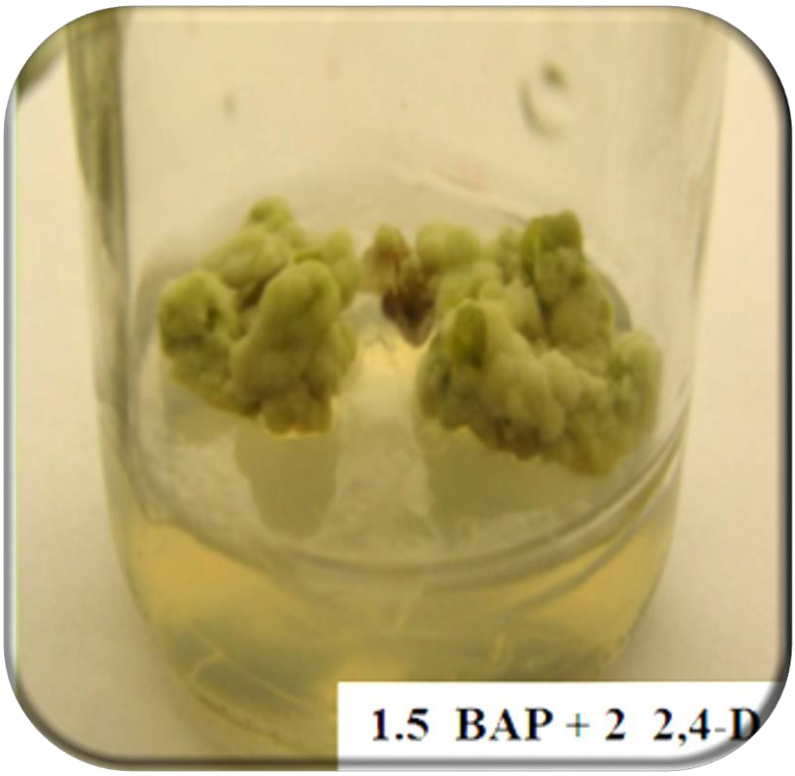

Figure 1. Formation of callus tissue on cotyledons grown on Whites- (WM) (1934) medium supplemented with 2 mg.L-12,4-D and 1.5 mg.L-1 BAP. *(photos were taken 21 days after Agriculture)*

b. Effect of adding some stimulants, chemical precursors, and abiotic stresses on callus growth indicators and the estimation of some nutrients in it.

Effect of Salicylic acid

The results of Table (2) showed that adding different concentrations of Salicylic acid had an effect on the studied traits. As the results showed a significant difference in the fresh callus weight, as the treatment of adding 50 mg L-1 of Salicylic acid was characterized by giving it the highest fresh callus weight of 7.08 g, while the control treatment gave the lowest fresh weight, amounting to 2.13 mg. As for the weight of dry callus adding salicylic acid has a great effect on it, as the treatment adding 50 mg L-1 of Salicylic acid gave it the highest dry weight of callus, amounting to 39.1 mg, while the control treatment achieved the lowest fresh weight, amounting to 0.11 mg. The results of the same table also indicated the effect of the concentrations of Salicylic acid added to the medium on the callus diameter, as the addition of 50 mg L-1 to the medium achieved the highest callus diameter of 12.34 mm, which differed

significantly from the rest of the addition., while the comparison treatment gave the lowest callus diameter, which reached 5.52 mm. It is also noted from the same table that there was a significant increase in the concentration of the nitrogen element, as the callus tissue responded to produce nitrogen at a concentration of 50 mg.l-1 of Salicylic acid in all treatments, as it gave the highest concentration of the nitrogen element, amounting to 0.97 µg.gm , which did not differ significantly from the 25 and 75 mg.L-1 treatments of Salicylic acid, which gave concentrations of 0.77 and 0.84 µg.gm. While the lowest concentration of nitrogen was reached in the comparison treatment. As for the concentration of the phosphorus element, we note from the same table that a significant difference appeared between the concentrations used, as the treatment of adding 50 mg.l-1 of Salicylic acid outperformed the rest of the concentrations and gave the highest concentration of the phosphorus element, amounting to 0.56 µg. gm , It did not differ significantly from the treatment of 75 mg l-1 of Salicylic acid, as it gave a concentration of the phosphorus element amounting to 0.48 µg.gm. as for the treatment adding a concentration of 25 mg l-1 of Salicylic acid and the comparison treatment gave the lowest concentration of phosphorus, which amounted to 0.31 and 0.20 micrograms.g-1. Straight dry weight. As for potassium, calcium, and sodium, they behaved the same way, as the results shown in the same table showed that the best increase in the concentration of potassium, calcium, and sodium occurred when a concentration of 75 mg.l-1 of Salicylic acid was added, as it gave an average of 0.98, 582.0, and 178.50 µg.gm respectively, which was significantly superior to the rest of the treatments, while the comparison treatment gave the lowest concentrations. While the concentration of 25 mg L-1 of Salicylic acid added to the nutritional medium gave the highest value of chloride, amounting to 70149 µg.gm dry weight which did not differ significantly from the 50 and 75 mg l-1 treatments, as they gave an average concentration of chloride amounting to 134.50 and 137.10 µg. gm in sequence, in When we notice that the concentration of chloride decreased significantly in the comparison treatment, reaching 117.00 µg.gm dry weight. Salicylic acid is an internal growth regulator and has significant effects on many vital processes that greatly affect plant growth during the various stages of its life [17][18][19]. Studies have indicated that moderate concentrations of salicylic acid Salicylates stimulate the division and growth of cultivated plant cells and tissues, as well as increasing their biomass and dry matter, depending on the plant type. The reason for the increase in the studied characteristics when adding Salicylic acid may be attributed to its effective role as it is one of the most important compounds of the new group Phytohormones, which regulate the growth of plant tissues and eliminate or reduce the damage of stress factors to which the plant is exposed. It also plays an effective role in inducing resistance to the harmful effects of cells growing under Tensile conditions [20] as explained by [21] Its contribution to the process of regulating all physiological processes such as nutrient absorption, protein synthesis, and inhibition of ethylene biosynthesis, as well as preventing the breakdown of the nucleus.

Table 2. effect of adding different concentrations of Salicylic acid on callus growth indicators of the belladonna plant Atropa belladonna L. and the estimation of some of its nutrients.

Salicylic acid mg.L	Fresh weight (g)	Dry weight (mg)	Callus diameter Mm)(	N µg. g	P µg. g	K µg. g	Ca µg. g	Na µg. g	Cl µg. g
0.0	2.13	0.11	5.52	0.62	0.20	0.42	137.50	64.00	117.00
25	4.11	1.28	8.32	0.77	0.31	0.45	378.00	153.50	149.70
50	7.08	3.91	12.34	0.97	0.56	0.77	412.50	162.00	134.50
75	5.59	2.50	7.95	0.84	0.48	0.98	582.00	178.50	137.10
L.S.D (0.05)	3.98 *	*1.69	*2.68	*0.20	*0.19	*0.33	*62.41	*22.04	*16.53

Effect of tyrosine acid

The results in Table (3) show that the concentrations of tyrosine acid added to the medium affected the fresh and dry weight and callus diameter, as the treatment of adding 60 mg.L-1 outperformed the rest of the treatments and gave the highest values in the fresh and dry callus weight and the relative growth rate reached 12.88 mg, 2.22 mg and 18.66 mm, respectively the lowest rates were for the control treatment which was given 12.88 mg, 2.22 mg, and 18.66 mm, respectively, while the comparison treatment gave less values. The added concentrations of tyrosine acid affected the ionic content of callus. Adding 20 mg.L-1 gave the highest value of nitrogen in callus tissues, reaching 0.91 µ g.g. The lowest concentration was for the comparison treatment, which amounted to 0.57 µg. g. Added concentrations of tyrosine did not significantly affect phosphorus and potassium levels. The same table showed that the concentration of the calcium element was affected significantly, as the concentration of 60 mg L-1 of tyrosine acid added to the nutritional medium gave the highest value of the calcium element, amounting to 498.50 µg.gm dry weight, while the lowest concentration of calcium was in the comparison treatment. As for the sodium element, it was also affected by the addition of tyrosine acid, as the 40 mg L-1 treatment gave the highest concentration of 185.30 µg .g. while control treatment

gave the lowest concentration of 72.00 µg.g. While chlorine was significantly affected by tyrosine the level of 60 mg L-1 gave the highest level of chlorine in callus tissues, which reached 129.00 µg.gm. The control treatment gave the lowest concentration of chlorine in the callus tissue which 101.00 µg. g. The reason for the results that appeared in Table (3) may be that the addition of the amino acid acid tyrosine has led to an increase in the concentration of plant hormones and enzymes, which acid tyrosine is part of, which in turn has led to an increase in biological activities, which is a common factor in them. within the plant, which was reflected in an increase in the studied traits [22].

Table 3. effect of adding different concentrations of tyrosine acid on callus growth indicators of the belladonna plant Atropa belladonna L. and estimation of some of its nutrients.

tyrosine acid -1mg.L	Fresh weight (g)	Dry weight (mg)	Callus diameter Mm)(	N µg. g	P µg. g	K µg. g	Ca µg. g	Na µg. g	Cl µg. g
0.0	3.43	0.09	6.56	0.57	0.21	0.60	157.50	72.00	101.00
20	7.64	1.01	10.75	0.91	0.28	0.65	275.00	137.50	114.30
40	8.91	1.61	13.82	0.90	0.31	0.71	389.00	185.30	117.00
60	12.88	2.22	18.66	0.87	0.25	0.68	498.50	177.90	129.00
L.S.D (0.05)	*2.69	*0.97	*3.06	*0.26	0.27ns	0.28 ns	*57.06	*48.13	*18.42

Addition effect of Jasmonic acid.

The results in Table (4) indicate the effect of the added concentrations of Jasmonic acid on the weight rate and callus diameter. As the results showed a significant superiority between the treatments, as the 50 mg.l-1 treatment was superior. 1 of Jasmonic acid for all treatments, which gave the highest average fresh and dry weight and callus diameter, which amounted to 13.51 mg , 2.32 mg and 11.82 mm, respectively, while there was a significant decrease in the fresh and dry weight and the diameter of the developing callus on the nutrient medium without any addition of Jasmonic acid, if the fresh weight reached 2.43 mg, 0.07 mg, and 6.41 mm,

respectively. Also The results shown in the same table indicate that there is a significant effect between the treatments on the concentration of nutrients, as the callus tissue responded to the production of nutrients when increasing Addition of Jasmonic acid to the comparison treatment, as the concentration of 75 mg L-1 of Jasmonic acid added to the nutrient media gave the highest concentration of the elements nitrogen, phosphorus, potassium, calcium, sodium and chloride if it reached 0.98, 0.57, 0.86, 637.00, 176.41 and 148.50 µg. g respectively while given treatment Comparison: The lowest rates were 0.74, 0.37, 0.52, 217.31, 66.00, and 125.00 µg. g , respectively. The increase in the studied characteristics when adding Jasmonic acid may be attributed to the role it plays in causing many physiological changes within plant tissues. Accompanied by morphological changes in these tissues, as well as a cycle in regulating production Plant cells for primary and secondary compounds by stimulating the expression of genes responsible for biosynthesis [23][24] or perhaps the reason is due to the effect of Jasmonic acid on regulating the work of cellular membranes and the permeable materials from them by regulating their entry according to the need for them. elements [25].

Table 4. effect of adding different concentrations of Jasmonic acid on callus growth indicators of the belladonna plant Atropa belladonna L. and estimation of some of its nutrients.

Jasmonic acid mg.L	Fresh weight (g)	Dry weight (mg)	Callus diameter Mm)(	N µg. g	P µg. g	K µg. g	Ca µg. g	Na µg. g	Cl µg. g
0.0	2.43	0.07	6.41	0.74	0.37	0.52	217.31	66.00	125.0
25	5.29	0.79	7.92	0.94	0.48	0.81	552.00	117.11	132.0
50	13.51	2.32	11.82	0.87	0.37	0.67	471.42	159.42	137.9
75	7.53	1.01	8.19	0.98	0.57	0.86	637.00	176.41	148.5
L.S.D (0.05)	2.47*	0.79*	*1.74	0.20*	0.18*	0.26*	102.47 *	35.64*	21.08*

Effect of PEG

The results in Table 5 indicate that adding PEG at different concentrations to the nutrient medium did not give a significant increase in the average fresh and dry weight and callus diameter, while the comparison treatment achieved the highest values in the mentioned characteristics, amounting to 4.55 g , 1.23 mg, and 6.03 mm, while the PEG addition treatment gave at a concentration of 1.5 g L-1, the lowest values for these characteristics were 2.10 g, 0.17 mg, and 3.88 mm. The results shown in the same table indicate a significant effect of adding different concentrations of PEG on the concentrations of elements in the callus tissue. The callus tissue responded to nitrogen production giving the highest significant increase of 80.97 µg.gm. At a concentration of 1.5 g.L-1 of PEG, which did not differ significantly from the treatment of adding 1.0 g.L-1 of PEG, which gave a nitrogen concentration of 0.91 µg.gm, The lowest concentration of the comparison treatment was 0.55 µg.g. As for the phosphorus element, the results in the same table showed that there was a significant difference between the concentrations of the water stress PEG used, as the treatment exceeded 0.5 g.l-1 of PEG and gave the highest concentration of the phosphorus element, amounting to 0.67 µg. g . While the comparison treatment gave the lowest values for the phosphorus concentration, which amounted to 0.31 µg. g . As for the potassium element, the results showed that the potassium concentration in the Callus was affected by added levels of PEG to the nutrient media as the 1.0 g.l-1 treatment outperformed the PEG gave the best increase in potassium concentration, which reached 0.96 µg. g , The lowest level of potassium ions was for the comparison treatment of 0.59 µg. g. It is noted from the statistical results shown in the same table that the concentrations of the calcium element were significantly affected when adding different concentrations of PEG, as the treatment of adding 1.5 g.l-1 of PEG to the nutrient media excelled and gave the highest concentration of calcium ion, which reached 611.50 µg. g with a significant difference from the rest of the concentrations, while the comparison treatment gave the lowest concentration of 347.50. µg. g. Likewise, the sodium element was also affected Through PEG levels added to the medium. The treatment of adding 1.0 g.l-1 of PEG was characterized by giving the best increase in sodium concentration, which reached 84.50 µg. g , which did not differ significantly from the 0.5 and 1 treatments. 5g.L-1 of PEG, while the control treatment gave the lowest sodium ion concentration of 66.40 µg. g. As for the concentration of the chloride element, the data showed that adding PEG to the nutrient medium led to increase in the concentration of the chloride element in the callus tissue, as the concentration of 0.5 gm.l-1 of PEG added to the nutrient medium gave the highest concentration. The chloride element reached 153.0 µg. g . Which did not differ significantly from the treatments of 1.0 and 1.5 g.L-1 of PEG. From the table we note that the comparison treatment gave the lowest chloride ion concentration, which amounted to 114.0 µg. g .The reason for the decrease in all levels of PEG8000 added to the nutrient media in terms of fresh and dry weight and callus diameter may be due to the accumulation of many compounds inside the plant when it is exposed to stress treatments, such as free radicals, which lead to high stress on the cells, which leads to plant weakness and lack of cell division [26] or it may be due to the cells losing their water content which negatively affected their growth, and the exposure of the plant to abiotic stresses leads to a reduction in Levels of endogenous plant hormones that encourage growth, such as auxins, cytokinins, and gibberellins, and raising the level of growth-inhibiting hormones, such as abscisic acid (ABA) [27] or perhaps a lack of water causes the accumulation of large quantities of organic solutions and mineral elements, And

as shown in the same table there is an increase in the concentration of elements. In the callus tissue, which may lead to the occurrence of a toxic effect that leads to inhibition of growth processes [28].

Table 5. effect of adding different concentrations of PEG on callus growth indicators of the belladonna plant Atropa belladonna L. and the estimation of some of its nutrients.

PEG g.L-1	Fresh weight (g)	Dry weight (mg)	Callus diameter Mm)(	N µg. g	P µg. g	K µg. g	Ca µg. g	Na µg. g	Cl µg. g
0.0	4.55	1.23	6.03	0.55	0.31	0.59	347.5	66.40	114.0
0.5	3.33	0.31	5.86	0.76	0.67	0.74	464.0	76.40	153.0
1.0	2.20	0.29	4.97	0.91	0.47	0.96	477.7	84.50	148.7
1.5	2.10	0.17	3.88	0.97	0.39	0.77	611.5	81.70	137.5
L.S.D (0.05)	2.37*	0.88	*2.27	0.24*	0.18*	0.30*	74.51*	16.49*	15.62*

Effect of NaCl.

The results in Table (6) show that there are significant differences between the treatments in fresh and dry weight and callus diameter, where the comparison treatment outperformed all treatments and gave 5.11 g, 2.62 mg, and 6.99 mm, respectively, while the treatment gave 75 mg.L-1, the lowest fresh and dry weight and diameter. For callus, it was 1.10 mg, 0.03 mg, and 4.57 mm, respectively. It is also noted from the table that salt stress negatively affected the nitrogen value in callus., The highest concentration was for the comparison treatment. 1.40 micrograms.gm-1., while the treatment of adding 25 mg L-1 of NaCl gave the lowest nitrogen concentration in callus, which reached 0.86µg.gm. While increasing the addition of concentrations of NaCl to the nutrient medium led to a gradual increase in the accumulation of phosphorus in the callus tissue, as the concentration of 75 mg.L-1 of NaCl gave the highest value of phosphorus, amounting to 3.41 µg.gm the lowest average was for the comparison treatment amounting to 0.21 µg.gm. As for the potassium element, differences appeared

between potassium concentrations as the comparison treatment outperformed the rest of the treatments and gave the highest value of the element, amounting to 2.61 µg.gm dry weight while the treatment gave 25 mg.l-1 the lowest concentration was 0.60 µg.gm dry weight. As for the calcium element, not affected by NaCl levels added to the media. The same argument shows that sodium and chlorine were affected by the levels of sodium chloride added to the medium, with a level of 75 mg/L giving the highest value of sodium and chlorine in callus tissue, which reached 187.0 µg.gm dry weight and 178.2 µg.gm dry weight respectively, while the control treatment gave the lowest concentrations of the two elements, amounting to 58.00 µg.gm dry weight and 109.1 µg.gm dry weight.The reason for the apparent effect of salt concentrations on the decrease in fresh and dry weight and callus diameter as a result of increased concentrations may be due to the variation in the osmotic potential inside the plant, which leads to the lack of access to the nutrients that the plant needs for growth and development, because the increase in salt concentrations leads to obstruction of tissue growth and thus a decrease in Average fresh and dry weight of callus. Salinity also has a negative effect on the nutritional balance of the plant through one or more mechanisms, including osmotic effects. Salts are involved in competitive absorption between ions in plant tissues, and increasing the salt level of the nutrient medium had the effect of reducing the amount of K+ as a result of the state of opposition between sodium and potassium ions, noting that sodium increases in concentration at the expense of potassium. The accumulation of Na+ in root cells caused an enzymatic disorder and negatively affected its active absorption [29].

Table 6. The effect of adding different concentrations of NaCl on callus growth indicators of the belladonna plant Atropa belladonna L. and estimation of some of its nutrients.

NaCl mg.L	Fresh weight (g)	Dry weight (mg)	Callus diameter Mm)(	N µg. g	P µg. g	K µg. g	Ca µg. g	Na µg. g	Cl µg. g
0.0	5.11	2.62	6.99	1.40	0.21	2.61	396.4	58.0	109.1
25	3.30	0.98	5.85	0.86	0.32	0.60	454.7	146.0	123.4
50	1.99	0.21	5.13	0.91	1.29	1.89	439.2	133.0	144.8
75	1.10	0.03	4.57	0.93	3.41	0.79	543.9	187.0	178.2
L.S.D (0.05)	2.08*	0.82*	*2.81	0.31*	0.73*	0.70*	62.6 ns	32.7*	7.81*

CONCLUSIONS

The study showed that growth regulators had an effect on the induction of callus from cotyledonous leaves, and that the addition of some stimulants, chemical initiators and abiotic stresses had a significant effect on the mineral content of the elements measured in the callus tissue.

REFERENCES

[1] Saad El-Din, Shurooq Mohammed Kazim, Adel Yousef Nasrallah, and Medhat Al-Sahouki. (2005). Effect of planting and transplanting dates on growth characteristics, yield, and belladonna alkaloids of Atropa belladonna.L. cultivated in open field, Iraqi Journal of Agricultural Sciences 80-75:(1)36.

[2] Al-Degwi, A. (1996). Encyclopedia of Medicinal and Aromatic Plants, Madbouly Library. Atlas Press (Book Two).

[3] Kayqon .(2008). Edible fruits in a cool climate the evalution and ecology of endozoochory in the European flora.In: furit and seed production : Aspects of development , Environmental physiolog and ecology (Society for Experimental Biology seminar series) Cambridge , UK: Cambridge university press pp. 240.

[4] Hopkins, W. G., & Hüner, N. P. (1995). Introduction to plant physiology.

[5] Ramawat , K.G. (2004). Plant biotechnology .Printed in New Delhi .India.p :50-62.

[6] Khan, T., Khan, T., Hano, C., & Abbasi, B. H. (2019). Effects of chitosan and salicylic acid on the production of pharmacologically attractive secondary metabolites in callus cultures of Fagonia indica. Industrial Crops and Products, 129, 525-535. https://doi.org/10.1016/j.indcrop.2018.12.048

[7] Musbah, H. M., Ibrahim, K. M., & Ibrahim, K. (2019). Effects of feeding tyrosine or phenylalanine on the accumulation of polyphenols in Coleus Blumei in Vivo and in Vitro. Journal of Biotechnology Research Center (JOBRC), 13(1), 35-43. https://doi.org/10.24126/jobrc.2019.13.1.566

[8] Adress, S., Khokhar, M. I., Fatima, R. N., & Rehman, S. U. (2024). In vitro effect of PEG and proline on callus growth and minerals values in basmati rice (Oryza sativa). Journal of Agricultural Sciences–Sri Lanka, 19(1). https://doi.org/10.4038/jas.v19i1.10147

[9] White, P. R. (1934). Potentially unlimited growth of excised tomato root tips in a liquid medium. Plant Physiology, 9(3), 585.

[10] Cresser, M. S., & Parsons, J. W. (1979). Sulphuric—Perchloric acid digestion of plant material for the determination of nitrogen, phosphorus, potassium, calcium and magnesium. Analytica Chimica Acta, 109(2), 431-436. https://doi.org/10.1016/S0003-2670(01)84273-2

[11] Jones Jr, J. B., & Case, V. W. (1990). Sampling, handling, and analyzing plant tissue samples. Soil testing and plant analysis, 3, 389-427. https://doi.org/10.2136/sssabookser3.3ed.c15

[12] Page, A. L. (Ed.). (1982). Methods of soil analysis. Part 2. Chemical and microbiological properties (pp. 1159-pp).

[13] Bhargava, B. S., & Raghupathi, H. B. (1993). Analysis of plant materials for macro and micronutrients. Methods of analysis of soils, plants, water and fertilizers, 49-82.

[14] Temminghoff, E. E., & Houba, V. J. (Eds.). (2004). Plant analysis procedures. Dordrecht: Springer Netherlands.

[15] Al-sahooke, M., & Waheeb, K. M. (1990). Applications in the design and analysis of experiments. Ministry of Higher Education and Scientific Research. Baghdad Univ. Iraq, 487.

[16] Chavan, J. J., Gaikwad, N. B., Umdale, S. D., Kshirsagar, P. R., Bhat, K. V., & Yadav, S. R. (2014). Efficiency of direct and indirect shoot organogenesis, molecular profiling, secondary metabolite production and antioxidant activity of micropropagated Ceropegia santapaui. Plant Growth Regulation, 72, 1-15. https://doi.org/10.1007/s10725-013-9830-7

[17] Khodary, S. E. A. (2004). Effect of salicylic acid on the growth, photosynthesis and carbohydrate metabolism in salt stressed maize plants. Int. J. Agric. Biol, 6(1), 5-8.

[18] Hayat, Q., Hayat, S., Irfan, M., & Ahmad, A. (2010). Effect of exogenous salicylic acid under changing environment: a review. Environmental and experimental botany, 68(1), 14-25. https://doi.org/10.1016/j.envexpbot.2009.08.005

[19] Babel, P., Devpura, V., & Purohit, S. D. (2014). Salicylic acid induced changes in growth and some biochemical characteristics in in vitro cultured shoots of Chlorophytum borivilianum sant. et. fernand. Int J Recent Sci Res, 5, 774-779.

[20] Gunes, A., Inal, A., Bagci, E. G., Coban, S., & Pilbeam, D. J. (2007). Silicon mediates changes to some physiological and enzymatic parameters symptomatic for oxidative stress in spinach (Spinacia oleracea L.) grown under B toxicity. Scientia Horticulturae, 113(2), 113-119. https://doi.org/10.1016/j.scienta.2007.03.009.

[21] Shakirova, F. M., Sakhabutdinova, A. R., Bezrukova, M. V., Fatkhutdinova, R. A., & Fatkhutdinova, D. R. (2003). Changes in the hormonal status of wheat seedlings induced by salicylic acid and salinity. Plant science, 164(3), 317-322. https://doi.org/10.1016/S0168-9452(02)00415-6.

[22] Maeda, H., & Dudareva, N. (2012). The shikimate pathway and aromatic amino acid biosynthesis in plants. Annual review of plant biology, 63(1), 73-105. https://doi.org/10.1146/annurev-arplant-042811-105439

[23] Ibrahim, E. A. (2016). Seed priming to alleviate salinity stress in germinating seeds. Journal of plant physiology, 192, 38-46. https://doi.org/10.1016/j.jplph.2015.12.011

[24] Nabi, N., Singh, S., & Saffeullah, P. (2021). Responses of in vitro cell cultures to elicitation: regulatory role of jasmonic acid and methyl jasmonate: a review. In Vitro Cellular & Developmental Biology-Plant, 57, 341-355. https://doi.org/10.1007/s11627-020-10140-6

[25] Lee, S. H., & Zwiazek, J. J. (2019). Regulation of water transport in Arabidopsis by methyl jasmonate. Plant Physiology and Biochemistry, 139, 540-547. https://doi.org/10.1016/j.plaphy.2019.04.023

[26] Herrera-Santoyo, J., López-Delgado, H., & Mora-Herrera, M. E. (2007). Stress in callus of Hippocratea excelsa: Catalase activity, hydrogen peroxide content and canophyllol accumulation. Interciencia, 32(4), 253-256.

[27] Farooq, M., Hussain, M., Wahid, A., & Siddique, K. H. M. (2012). Drought stress in plants: an overview. Plant responses to drought stress: from morphological to molecular features. International Journal of Agriculture and Biology, 11, 100-105.

[28] Errabii, T., Gandonou, C. B., Essalmani, H., Abrini, J., Idaomar, M., & Skali-Senhaji, N. (2006). Growth, proline and ion accumulation in sugarcane callus cultures under drought-induced osmotic stress and its subsequent relief. African Journal of Biotechnology, 5(16).

[29] Mengel, K.and Kirkby, E. A. (2001). Principles of plant nutrition, 5th Edu (Kluwer Academic Publishers: Dordrecht,The Netherlands).

***Conflict of Interest Statement**:The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.*