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EFFECT OF GROWTH REGULATORS (NAA AND BA) ON CALLUS FROM MORINGA OLEIFERA L. IN VITRO.

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Abstract:

Effect of growth regulators (BA) - (IAA-BA) - (NAA) at concentrations (0-0.5-1.0-1.5-2) mg/L of callus produced from (2,4-D-BA) produced from (Moringa oleifera L.) in vitro. Where there was a significant effect of the duration of sterilization in reducing the percentage of pollution, which reached the lowest value of 22.50% at the time period of 20 minutes. As for the concentration, we note that the 20% concentration gave the lowest contamination rate, which amounted to 58.50%, which was significantly excelled on all other concentrations. As for the interaction between the concentrations and the time of treatment, 20 with concentration 20, as the lowest contamination rate reached 15%. Also, there was a significant effect of the interaction between the concentrations of auxin and cytokinin in the effect on the fresh weight of the callus. The treatment (4) of 2,4-D at a concentration of (1.5) mg/L interaction with BA at a concentration of (2) mg/l-1 in giving The highest callus fresh weight was 1.45 mg and the lowest was when the control treatment was 0 from 2,4-D × BA It reached (1.04) mg. Also, the interaction between the concentrations of auxin and cytokinin was significant in affecting the fresh weight of the callus, and treatment (3) of IAA with a concentration of (1.0) mg/L interaction with BA at a concentration of (0.5) mg/L^{-1} excelled in giving the highest fresh weight of the callus. It reached (830 mg), and the lowest treatment is when the control treatment is 0 from NAA x BA it reached 238 mg.

Keywords: Moringa oleifera L. - callus - MS - growth regulators

Introduction:

The Moringa tree, the miracle tree, or the daisy tree Moringa oleifera L. belongs to Moringaceae family , and the English name is Drum sticks. They are grown as ornamental trees for the beauty of their flowers and abundant shade, or to make a plant fence or as a windbreak (Poteet, 2006). Therefore, all parts of the tree are of nutritional and medical importance. The plant constitutes an integrated food in some regions of Asia and Africa. The leaves are used as a dietary supplement for people with immunodeficiency because they contain large amounts of vitamins and amino acids.carbohydrates, iron, potassium, phosphorous, calcium, selenium, zinc and antioxidants (Farooq et al., 2012; Yadav and Srivastava, 2016). It is native to South and East Asia and spread wildly in the Himalayas (Northwest India), from which it spread to all parts of the world (Devendra et al., 2012). The Morinca plant is rich in some compounds, such as: Zeatin, a plant hormone that belongs to the group of cytokinins. It is an anti-aging compound that is important in cell division and contains a number of compounds of medical importance such as β-sitosterol, caffeoylquinic acid, quercetin, and kaempferol, and these two The other two compounds belong to the group of phenols, which are of importance to plants and humans, which reduce the risk of cancer and some heart diseases. They act as antioxidants and antibiotics, against fungi and pathogenic bacteria (Makonnen et al., 1997 and Abdulkarim et al., 2005). The researchers were able to propagating

plants invitro and the applied studies tended to use tissue culture technology, to propagating and produce large numbers of plants similar to the mother plant in a relatively short time and throughout the year and in small areas (Fahmy, 2003 and Al-Sumaidaie, 2017). The callus is defined as a group of undifferentiated parenchymal cells that arise in the areas of cuts or wounds in explant. The process of inducing callus on the explant cultured in the nutrient medium is one of the basic and important processes, and the process of inducing callus goes through three stages: the stage of stimulation, the stage of division, and the stage of Differentiation. The first stage is in which several changes occur in the cells to be prepared for the process of division, as well as the construction of proteins and DNA replication. In the second stage, cell division occurs sequentially to obtain a mass of callus cells, and in the third stage, the meristematic cells expand and differentiate to obtain the appropriate growth factors. There are several factors that affect the induction of callus, including genetic factors and environmental factors, including light, heat, and humidity, and there are chemical factors such as nutrients as well as growth regulators in the medium (Al-Rifai and Al-Shweiki, 2007) and the callus cells have the ability to form structures and organs that will give in the future a whole plant or have the ability to produce secondary metabolic compounds and there is some explant that does not have the ability to form callus (Smith, 2000).The external appearance and the cellular composition of the callus tissues differ according to the type of plant. It may be solid or brittle in texture or it may be colorless, white, brown, yellow or violet depending on the presence or absence of plant pigments (Dodds and Roberts, 1995 and Fahmy, 2003).

Materials and methods

The study was conducted in the plant tissue culture laboratory of the Department of Plant Production - Postgraduate Studies - Al-Musauib Technical College - Al-Furat Al-Awsat University for the period from 1/11/2021 to 1/5/2022. To study the effect of some plant growth regulators: (BA--NAA) and (IAA-BA) at concentrations (0.0-1-0.5-1.5-2) mg L^{-1}) on callus resulting from the effect of (2,4-D-BA) of Morinca plant. in vitro study of the effect of these regulators on the production of callus Madame

1- The culture medium

I used the ready-made nutrient medium produced by Murashige Company (HIMEDIA and Skoog, 1962), and then I prepared the MS medium for growing explant. I weighed 4.91 $g.L^{-1}$ of the ready-made powder to prepare one liter of distilled water and added agar agar at a concentration of 7g.L. $^{-1}$ Sucrose was added at a concentration of 30 $g.L^{-1}$, and the pH ranged from 5.6 to 5.8, by adding drops of one standard solution of sodium hydroxide (NaOH) or hydrochloric acid (HCl).The components of the nutrient medium are heated on the Hot Plate Magnetic Stirrer for the purpose of dissolving the components of the nutrient medium, and after the medium has become homogeneous, I distribute it in tubes for cultivation, which are glass containing MS medium at an amount of 10 ml per tube, and sterilized with an Autoclave at a temperature of 121 °C. ° and a pressure of 1.04 kg. cm^2 for 20 minutes. The tubes were removed from the autoclave after the end of the sterilization period and left to harden at room temperature.

2- Sterilization:

2-1 Sterilization of work tools and explants

All the tools used in the cultivation process, including scalpels, tweezers, and glassware, were sterilized after covering them with aluminum foil, and they were sterilized with an autoclave at a temperature of 121 °C and a pressure of 1.04 kg. A temperature of 40 ° C for 12 hours, and after that, when planting, we enter all the cultivation requirements inside the previously sterilized planting cabin using ethanol alcohol with a concentration of (70%) and using ultraviolet (UV) ultra violet rays half an hour before the planting process, We sterilize the tweezers and blades before starting the cultivation process using ethyl alcohol at a concentration of 95%, and we also wear a mask and a laboratory vest, and we use ethyl alcohol at a concentration of 70% to sterilize hands. We remove the plant parts obtained from the seedlings and cut them into small parts with a length of 3 cm or 4 cm and sterilize the plant parts with commercial bleach with a concentration of 6% of sodium hypochlorite NaOCl at concentrations (0-10-15-20)% and for periods (5-10- 15-20) minutes.

1- Induction of callus

3-1 formation of callus cultures by studying the effect of different concentrations of 2,4-D and BA

After obtaining (growing tops - new branches - and leaves) from which callus is to be induced, these parts were planted in MS medium, which contains different combinations of growth regulators including 2,4-D - BA in concentrations (0, 0.5, 1.0, 1.5, 2.0) (0, 1.0, 0.5, 1.5, 2.0) mg.L-1, sequentially, with ten replicates for each concentration, and explants were incubated to induce callus in complete darkness under the temperature of 25 °C ± 2 with light for 16 hours and 8 hours of darkness. Under the light intensity of 1000, the results were recorded after six weeks of cultuer and the completion of callus growth.

3-2 Maintaining the callus produced from the previous experiment by culturing it on a medium containing (NAA-BA)

A subculture was performed for the induced callus from the previous experiment, where the callus was cut, and the damaged parts were sorted and excluded using tweezers and sterile blades. Then, they were grown on MS medium containing different concentrations of (NAA-BA). The concentrations were (0-0.5-1.0-1.5-2.0) $mg.L^{-1}$. The cultured callus was transferred to the growth chamber at a temperature of 25 ± 2 °C. Under conditions of complete darkness, after 45 days, the fresh weight of the formed callus is measured and transferred to the analysis.

2- Results and discussion:

4-1: The Effect of Sodium Hypochlorite Concentrations and the Sterilization Period on the Percentage of Pollution of the Growing Tops and Leaves of the Morinca Plant

Table (1) shows the effect of sodium hypochlorite concentrations used and their interaction with the sterilization period in the percentage of reducing the pollution percentage of the growing tops and leaves of the Morinca plant. Concentration We note that the 20% concentration gave the lowest contamination rate, which amounted to 58.50%, which was significantly superior to all other concentrations. As for the overlap between the concentrations and the time of treatment, 20 with concentration 20, as the lowest contamination rate reached 15%. The reason is due to the effect of

sodium hypochlorite, and its work as a basic and sterilizing substance for plant tissues. The reason is due to Hypochlorous Acid (HOCL), which is considered as a strong oxidizing substance.The acid is formed as a result of chlorine dissolving in water: (2004, Ramawt)

Table (1) The effect of sodium hypochlorite concentrations and the sterilization period on the percentage of contamination of the growing tops and leaves after 15 days of culture

average	20	15	10	0	concentration / time
100	100	100	100	100	**5**
89	76	80	100	100	**10**
51	43	46	55	60	**15**
22.50	15	20	25	30	**20**
	58.50	61.50	70.00	72.50	**average**
1.315 concentration= * time		**0.657 concentration=**		**0.657 = time**	**L.S.D 0.05**

4-2 Effect of 2,4-D and BA and the interaction between them on the fresh weight of callus induced from explant cultured in MS medium after 30 days from the date of culture.

Table (2) shows The interaction between the concentrations of auxin and cytokinin was significant in affecting the fresh weight of the callus, and treatment (4) of 2,4-D at a concentration of (1.5) mg/L^{-1} was excelled in interaction with BA at a concentration of (2) mg/L. L^{-1} gave the highest fresh weight of callus (1.45 mg).The lowest treatment is when the control treatment is 0 of 2.4-D x BA reaches (1.04) mg. These results are consistent with what previous studies indicated that the medium that contains plant growth regulators leads to the growth and expansion of embryonic nodes, and their rapid development into embryos, and this leads to the continuation of the division of callus cells and not giving it vegetative embryos (Saad, 2001).

Table (2) Effect of 2,4-D and BA and the interaction between them on the fresh weight of callus induced from explants cultured in MS medium after 30 days from the date of culture.

average	2	1.5	1	0.5	0	BA / 2,4-D
1.21	1.17	1.16	1.15	1.14	1.04	**0.0**
1.20	1.37	1.36	1.35	1.16	1.25	**0.5**
1.24	1.23	1.27	1.26	1.26	1.26	**1.0**
1.25	1.45	1.39	1.39	1.38	1.36	**1.5**
1.28	1.18	1.06	1.05	1.05	1.14	**2.0**
	1.08	1.39	1.26	1.30	1.15	**average**

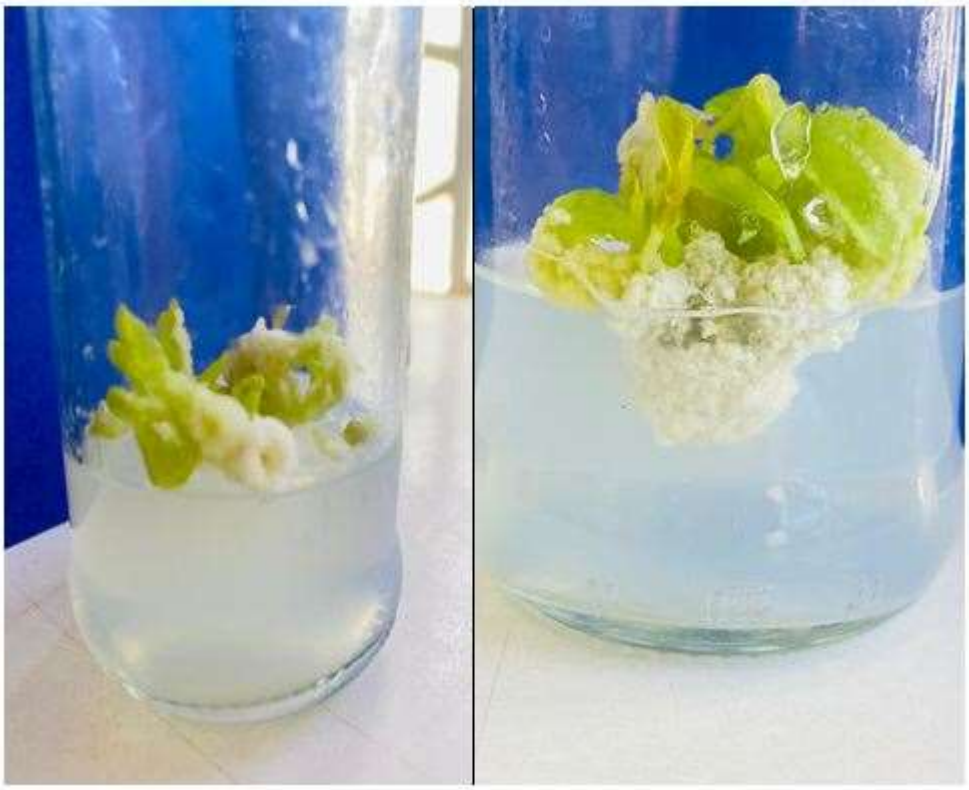
BA= 0.25 2,4-D 0.25 BA *2,4-D = 0.56	**L.S.D 0.05**

4-3 Effect of growth regulators (NAA-BA) mg-l^{-1} on the fresh weight of callus (sub culture) after 45 days after culture, and 200 mg of callus was taken.

Table (3) shows and indicates that the interaction between the concentrations of auxin and cytokinin was significant in affecting the fresh weight of the callus, and treatment (3) of IAA at a concentration of (1.0) mg/L^{-1} overlapped with BA at a concentration of (0.5) mg/L^{-1} in giving The highest fresh weight of the callus was (830 mg), and the lowest treatment was when the control treatment was 0 of NAA × BA if it reached 238 mg. These results are consistent with what previous studies indicated that the presence of auxins and cytokinins in the nutritional medium has a role in inducing callus and increasing its growth, and encourages The processes of cell division and growth with an increase in the manufacture of basic contents to increase division and growth, such as amino acids and proteins, and lead to an increase in the biomass of the callus and then an increase in the rates of fresh weight. (Al-Mahdawi, 2016) and (AL-Jibouri et al., 2016).

Table (7) table of the effect of growth regulators (NAA-BA) mg-l-1 on the fresh weight of callus (sub culture) after 45 days after replanting, and the weight of 200 mg of callus was taken.

average	2	1.5	1	0.5	0	BA / NAA
290.80	332	341	290	253	238	**0**
392.40	347	523	338	467	287	**0.5**
542.20	431	432	576	830	442	**1**
492.67	328	496	734	524	381	**1.5**
491.00	351	448	557	672	427	**2**
	357.80	448.07	499.00	549.20	355.00	**average**
IAA*BA = 1.618 BA = 0.724 NAA = 0.724						**L.S.D 0.05**



Figure(1) Moringa callus

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