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Propagation of *Nephrolepis exaltata* L. in vitro

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Abstract

This experiment was conducted in the plant tissue culture laboratory of the Department of Plant Production Technologies / Al-Musayyib Technical College / Al-Furat Al-Awsat Technical University from 9/1/2022 to 6/1/2023 to study the effect of some plant growth regulators: auxin (acetic acid (2,4- Dichlorophenoxy (in concentrations of 0.0, 1, 2, 3, and 4 mg.L^{-1}) and cytokinin (Benzyle adenine) in concentrations of 0.0, 0.5, 1, 1.5, and 2 mg.L^{-1} and their interactions in the propagation of Nephrolepis exaltata plants in vitro and their effect on callus induction, callus growth, and vegetative shoot generation and root group, as well as fresh weight and dry weight. The known nutrient medium was used (Murashig and Skoog, 1962). This experiment was designed according to a completely randomized design (CRD) with five replicates, then the means were compared using the least significant difference (LSD) under the probability level of 0.05. The results showed excelled The concentration of 3.0 mg.L showed a significant effect on all treatments, giving it the highest percentage of 53.3%, followed by the concentration of 2.0 mg.L (46.7%), while the comparison percentage was at the lowest value of 6.7%. While the growth regulator BA had a significant effect on The percentage of callus induction increased as the concentrations 2 mg/L and 1.5 mg/L. liter excelled on the rest of the concentrations, giving them the highest percentage of callus induction, amounting to 53.3 and 40%, respectively, while the control treatment gave the lowest percentage of callus induction, amounting to 13.3%. As for the number of shoots, the concentration of 1.5 mg/L gave an increase in the average number of shoots by 4.62 shoots, which was significantly excelled on the control treatment (3.37 shoots). The growth regulator BA, where the concentration of 2 mg/L was significantly excelled on some concentrations in the number of shoots, gave it the highest rate. It reached 5.29 shoots per explant. As for the rooting stage, NAA at a concentration of 1.0 mg/L exceeded the percentage of rooting, which amounted to 57.00%. And the concentration of 2.0 mg/L excelled on the average number of roots and their lengths, as it gave 2.98 roots with a length of 3.14 cm, while the comparison treatment and concentration did not give any results. 0.5 mg.L^{-1}, any rate.

Keywords: NAA, BA, 2-4-D

introduction

Nephrolepis exaltata L is a fern plant that belongs to the ephrolepidaceae family, and is native to North, Central, and South America. It is known as the sword fern or Boston fern. It is one of the most common foliage plants. It is grown for indoor beautification due to the beauty of the long, feathery leaves, which are small leaflets on each side. It is a perennial fern with a length of 50-150 cm. It is widely used in homes. It is raised in hanging plants and grown in baskets or in a symmetrical manner and in pots. It grows best in humid conditions and outdoors, although this plant tolerates full or partial shade. Due to its enhanced ornamental value and higher tolerance to indoor environmental conditions, the mutant was named N. exaltata 'Bostoniensis' and quickly gained popularity as Boston (1). General Nephrolepis' dark green foliage with long-lasting properties is commonly used in the floral industry around the world (2). Nephrolepis is propagated by dividing the plant (lobbing), by removing the plant from the pot and separating the seedlings from the mother plant with a sharp, sterile knife, so that each seedling retains roots and leafy stems and is planted in a separate place. The best time for propagation is spring to early summer. - It can also be propagated with spores and grown in moist soil at a temperature of 21 degrees Celsius, and it must be planted immediately after harvesting it from the plant. Researchers were able to propagate plants in vitro, as applied studies tended to use tissue culture technology, to propagate and produce large numbers of plants similar to the mother plant at a time. Relatively short, throughout the year in small areas (A3), plant tissue culture includes all life techniques through which a tissue, cell or plant organ is removed or isolated under conditions free of pathogens, sterilized, and then grown in sterile artificial nutritional media. Also, incubating the planted part in specific environmental conditions in terms of light and temperature, with the aim of directing the planted explant in the direction required for its culture (4).Growth regulators are among the most important factors in tissue culture added to the nutrient medium for the success of the micropropagation process of plants, and auxins and cytokines are among the most widely used regulators for this (5). Agriculture aims to:

1 Plant propagation in vitro.

2. Study the effect of auxins and cytokines on the Nephrolepis exaltata plant.

Materials and methods

This experiment was conducted in the plant tissue culture laboratory of the Department of Plant Production Technologies / Al-Musayyib Technical College / Al-Furat Al-Awsat Technical University from 9/1/2022 to 6/1/2023. The study studied the effect of some plant growth regulators: such as auxin (Naphthalene acetic acid) at concentrations 0.0 and 1, 2, 3, and 4 mg/L and cytokinin (Benzyle adenine) at concentrations of 0.0, 0.5, 1, 1.5, and 2 mg/L and their interactions in the propagation of Nephrolepis exaltata plants ex vivo, as well as fresh weight and dry weight. The known nutrient medium was used (10). The explant of the Nephrolepis exaltata plant were taken and placed in a laboratory beaker with a capacity of 250 ml. They were washed with water and liquid soap, then they were placed under running tap water for one hour to remove dust from them. Then they were transferred to the Laminar air flow cabinet to perform the sterilization process, where they were surface sterilized with different concentrations. of sodium hypochlorate NaOCI for the purpose of sterilizing explant and concentrations (0, 2, 4, 6) for (5, 10, 15, 20) minutes and then transferred to a 250 ml glass container containing ethyl alcohol at a concentration of 70% for a minute and then Then it was washed with sterile distilled water three times to remove the remaining sterilizing materials. After that, it was placed in Petri dishes that had previously been sterilized with alcohol and burned with flame. It was divided into parts as in Figure (1), and grown on MS medium and incubated in the growth room at a temperature of 25 + 2 and a light intensity of 1000. 1 lux (16)

hours of light followed by 8 hours of darkness alternately, and the results were obtained two weeks after culture.

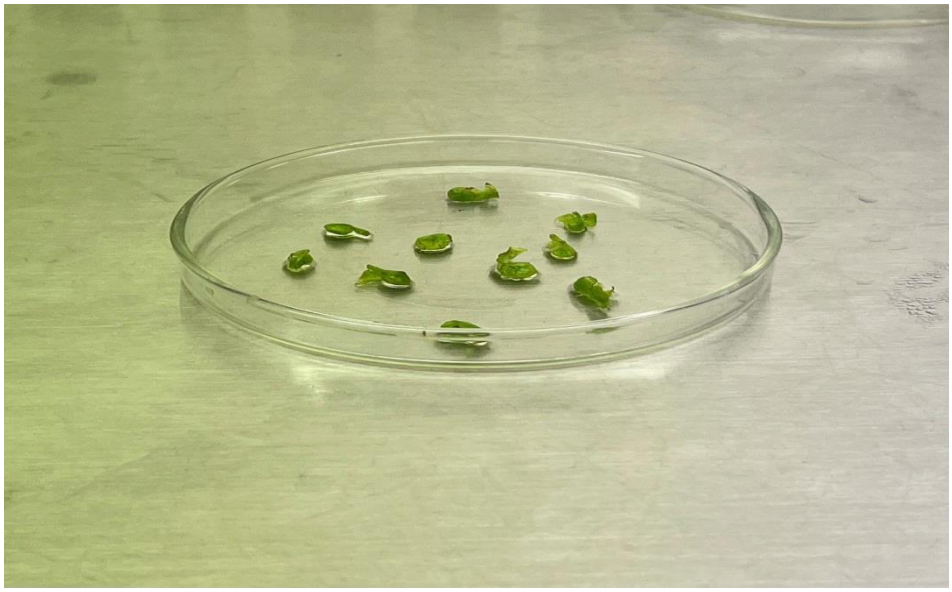


Figure (1) explant prepared for culture

4.49 grams of the ready-made powder for the nutrient medium was weighed to prepare a liter of food explant, and agar was added to it as a solidifying substance for the nutrient media in the amount of 7 g/liter after adding sucrose 3%, myo-inositol (Monositol 100 mg/liter), BA and D-2.4 according to the requirements of the experiment. Adjust the mean to 6. 5 By adding hydrochloric acid and (HCI) or sodium hydroxide solution (NaOH), and cooking the medium by placing it on a hot plate magnetic stirrer, then pouring it into test tubes at a rate of 10 ml for each tube and after closing them tightly, place it in an autoclave at 100°C. A temperature of 121°C and a pressure of 1.04 kg/cm for 15 minutes, then it was taken out of the incubator and left to cool and the medium solidified at room temperature, thus making it ready for culture. The experiment was conducted to find out The experiment was conducted to know the effect of 2-4-D and BA (mg L-') and their interaction in growing Nephrolepis exaltata explant in nutrient media. The plant parts were grown on sterile media (Murashige, and Skoog, 1962) provided with different concentrations of regulators. Growth 2-4-D(0.0, 1, 2, 3, 4) mg L^{-1} and BA (0.0, 0.5, 1.0, 1.5, 2) mg L^{-1} and five replicates.

Effect of D-4,2 and BA on the percentage of callus induction from the growing apex after 6 weeks of culture

Table (2) shows that there was a significant effect of the growth regulator D,2,4- in increasing the percentage rate of callus induction from the growing apex of the Nephrolepis exaltata plant after six weeks of culture, where the concentration of 3.0 mg/L significantly outperformed all treatments by giving it the highest percentage of 53 3%, followed by 2.0 mg at that concentration." (46.7%), while the comparison percentage was at the lowest value of 6.7%

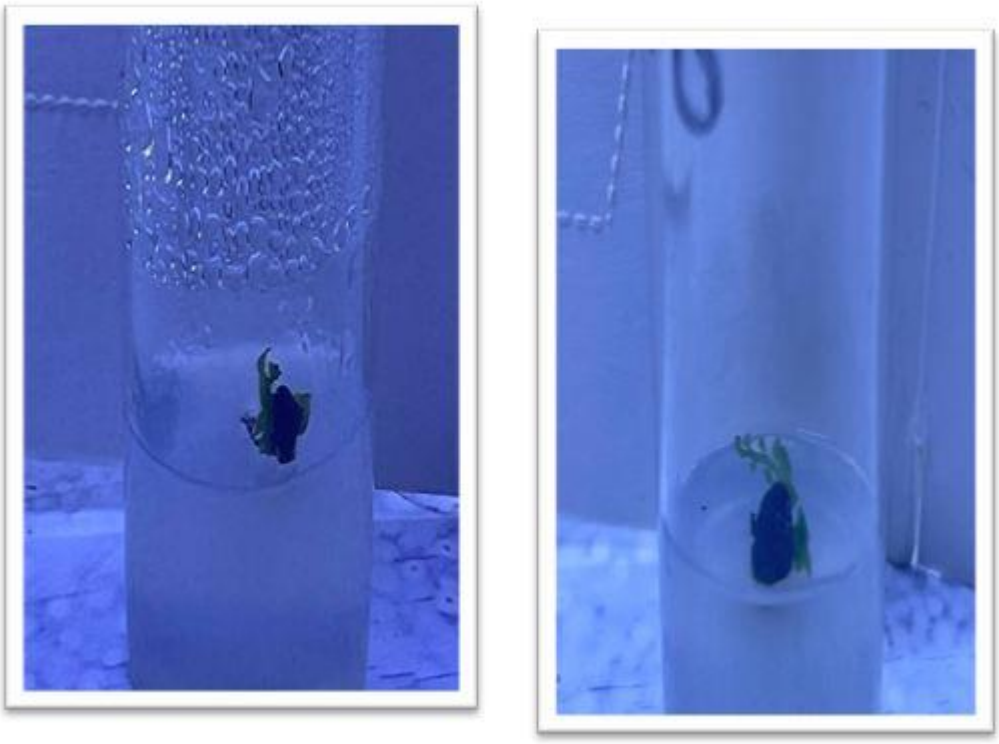
The results of the same table also showed that the use of the growth regulator BA had a significant effect in increasing the percentage of callus induction, where the two concentrations 2 mg/L and 1.5 mg/L. liter excelled on the rest of the concentrations, giving them the highest percentage of callus induction, amounting to 53.3 and 40%, respectively, while the control treatment gave the lowest percentage of callus induction, amounting to 13.3%. Previous studies have shown that the ability of plant parts to form callus increases when the nutrient medium is prepared with growth regulators such as cytokinins and auxins, as their interaction in different concentrations is important for the formation of callus and its growth in order to achieve an ideal compatibility in the internal hormonal status of the plant part compared to using them alone (6 and 7).

The results of the same table showed that there was a significant effect of the interaction between the growth regulators D-2,4 and AB in increasing the percentage of callus induction, as most of the interaction combinations, 3 of D-2,4 and 2 of BA, gave the highest values, amounting to 100%, and without significant differences. between them, while no control treatment was given. Cytokinins are used in low concentrations to produce physiological effects in the cultivated plant part, and in balance with auxins, they help in inducing callus (8). Many researchers have indicated the possibility of inducing callus depending on the plant part. Growth regulators added to the nutrient medium and a balanced combination increase the power of callus stimulation (9))

It has been shown from this result that the relationship between the concentration and quality of the growth regulator and the ratio between them is the main factor in the process of inducing callus from plant tissues (10). In previous years, studies have developed in the field of callus induction and its use in the production of secondary metabolic compounds from medically important plants and comparing them with the original plant or plant growing in the field, as these compounds are considered to have high biological effectiveness and are highly stable compared to artificially produced compounds (11).

Table (1) Effect of D-4,2 and BA on the percentage of callus induction from the growing apex after 6 weeks of culture.

average2-4-D	BA					2،4-D
	2	1.5	1	0.5	0	
6.7	33.3	0	0	0	0	0
13.3	33.3	33.33	0	0	0	1
46.7	66.7	66.7	33.33	33.33	33.3	2
53.3	100	66.7	33.33	33.33	33.3	3
13.3	33.3	33.3	0	0	0	4
	53.3	40	13.3	13.3	13.3	average BA
	70.86=interaction			31.69= BA	31.69=2، 4-D	L.S.D 0.05



Callus induction after 45 days of culture the vegetative part of Nephrolepis exaltata in the nutrient medium prepared with a concentration of D4,2-3.0 and BA 2.0.

The effect of NAA and BA and their interaction on the number of shoots 30 days after culture

The results of Table (2) showed that there were significant differences between the concentrations of NAA, as all the concentrations of NAA used were significantly excelled on the control treatment. The concentration of 1.5 mg/L gave an increase in the average number of shoots, amounting to 4.62 shoots, which was significantly excelled on the control treatment (3.37 shoots).

We note from the same table that there is a significant effect of the growth regulator BA, as the concentration of 2 mg/L was significantly excelled on some concentrations in the number of shoots by giving it the highest rate of 5.29 shoots per plant part. The reason for the increase in the rate of the number of vegetative shoots may be due to the cooperative action between cytokinin and auxin in encouraging vegetative growth in appropriate concentrations. These results are consistent with what was found by (12a), who stated that the interaction between auxins and cytokinins at appropriate concentrations leads to encouraging the growth of vegetative shoots and increasing their number. (13) explained that cytokinins play an important role in overcoming apical dominance and stimulate the formation of axillary buds, thus leading to an increase in the number of shoots per plant. It is believed that cytokinin stimulates the formation of woody tissue in the buds and stem, which facilitates the transfer of water and nutrients and thus the lateral bud grows (14). (15 and 16) indicated that the importance of adding cytokinin to the nutrient medium in the multiplication stage lies in helping the plant part to grow and pushing it to form a vegetative branch. By balancing with the internal auxins produced by the plant part, which works on the formation of adventitious shoots, which help in their growth.The results of the table also showed that there were significant differences when BA and NAA were intermingled, as the concentration of 2 mg/L of BA and the concentration of 0.5 mg/L of NAA were excelled, giving them the highest value in the number of shoots, reaching 7.5 shoots, while the concentration of 0 mg/L of BA and 1 mg/L of NAA gave The lowest value in the number of shoots was 0.15 shoots, while the control treatment gave 0.53 plant shoots. We note from the results shown that the number of plants per plant of Nephrolepis exaltata

increases with increasing concentrations of NAA and BA. This may be due to the role of cytokinins as a plant growth regulator that causes bud stimulation by stimulating cell division and reducing apical dominance (17), while NAA works to increase cell division (18).)

Table (2) Effect of NAA and BA and their interaction on the number of shoots 30 days after culture

NAA average	BA					NAA
	2.5	2	1.5	1	0	
3.37	6.23	4.22	1.56	4.31	0.53	0
4.11	6.3	7.5	3.47	2.68	0.64	0.5
3.92	4.88	4.98	4.92	4.70	0.15	1
4.62	6.10	5.2	5.96	5.11	0.74	1.5
3.56	2.10	4.55	6.5	3.62	1.04	2
	5.12	5.29	4.48	4.08	0.62	average BA
	.00937=interaction			.004193 BA	.004193 NAA	.S.D 0.05

The effect of NAA and BA and their interaction on the number of shoots after 30 days of vegetative culture of Nephrolepis exaltata on prepared medium with a concentration of .50 NAA and 2.0 concentrations of BA.

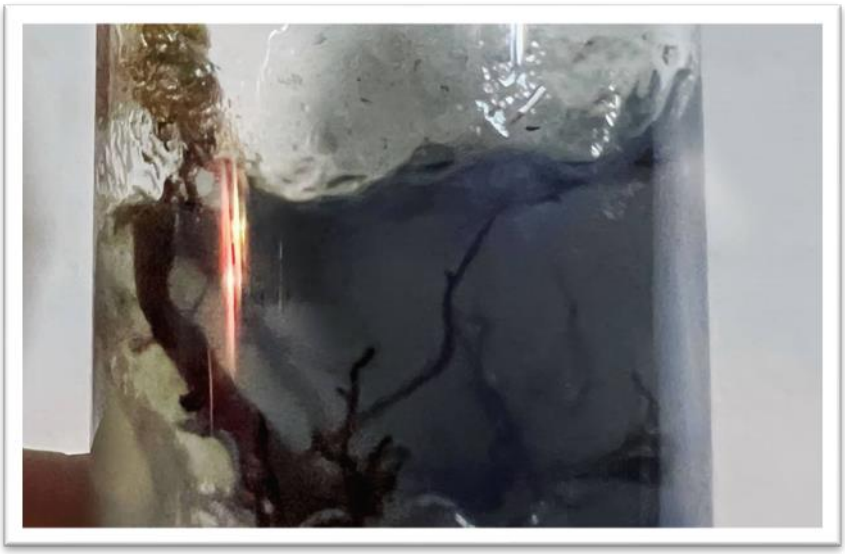
Effect of NAA concentrations in MS medium with half the strength of its salts on the percentage of Nephrolepis exaltata plants and the average number and length of roots after 30 days of culture in the rooting medium.

It was found that using the MS nutrient medium with full salt strength did not give any rooting rate at all concentrations. The results shown in a table indicate that NAA at a concentration of 1.0

mg/L excelled on the percentage of rooting, which amounted to 57.00% compared to the medium devoid of auxins and the rest of the concentrations, while the control treatment and the treatment with a concentration of 0.5 mg/L did not give any percentage according to the conditions of the experiment. The same table also showed that the concentration of 2.0 mg/L was superior in the average number of roots and their length, as it gave 2.98 roots with a length of 3.14 cm, while the control treatment and the concentration of 0.5 mg did not give any results. liter any rate. These results are consistent with (19) when adding 2 mg/L to the medium containing half the strength of salts, which gave a rooting rate of 100%. Also, the role of auxins in stimulating cell division and the presence of internal concentrations of cytokinins that came from the replicaten stage, in addition to the elongation and cell expansion that This is due to the fact that auxin increases the elasticity and plasticity of the cell wall, thus reducing the wall's resistance to tension. This leads to the cell wall responding to osmotic pressure as a result of the presence of dissolved substances, or in other words, an increase in dissolved osmotic substances in the cell juice (21).

Table (3)ffect of NAA concentrations in MS medium with half the strength of its salts on the percentage of Nephrolepis exaltata plants and the average number and length of roots after 30 days of culture in the rooting medium.

Average root (length (cm	Average number of roots	Rooting percentage	(mg.l^{-1})NAA
0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.5
2.64	2.75	57.00	1.0
2.86	2.85	1.93	1.5
3.14	2.98	35.00	2
0.033	0.040	1.152	L.S.D0.05



The effect of NAA concentrations in MS medium with half the strength of its salts on the percentage of Nephrolepis exaltata plants and the average number and length of roots after 30 days of culture in the rooting medium.

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