# Callus Production by *Nephroleips exltata* L. Plant in Vitro

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**Abstract:**
This experiment was conducted in the plant tissue culture laboratory at 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 (at concentrations of 0.0, 1, 2, 3, and 4 mg.$L^{-1}$) and cytokinin (Benzyle adenine) at concentrations of 0.0, 0.5, 1, 1.5, and 2 mg.$L^{-1}$ and their interactions in the propagation of Nephrolepis exaltata plants ex vivo and their effect on callus induction, callus growth, vegetative shoot generation, and shoot formation. Root weight, fresh weight and dry weight. The well-known nutrient medium (Murashig and Skoog, 1962) was used. 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 that the percentage The contamination rate was 100% in the control treatment, and the contamination rate decreased with increasing sterilization duration. The contamination rate reached 63.25% at 20 minutes on the other hand, the concentration of 4% gave the lowest contamination rate, amounting to 28.25%. As for the callus induction stage, the concentration of 3.0 mg .$L^{-1}$ significantly excelled In all treatments, the highest percentage was given at 53.3%, followed by 2.0 mg at that concentration. L (46.7%), while the comparison percentage was at the lowest value of 6.7%. While the growth regulator BA had a significant effect in increasing the percentage of callus induction, as the two concentrations, 2 mg.$L^{-1}$ and 1.5 mg.$L^{-1}$ excelled on the rest of the concentrations by giving them the highest percentage. The percentage of callus induction reached 53.3 and 40%, respectively, while the control treatment gave the lowest percentage of callus induction, amounting to 13.3%. While in fresh weight, the concentration of 3 mg. liter of 2,4-D gave the highest rate of fresh weight, which reached 0.52 mg. While the control treatment gave the lowest fresh weight of 0.01 mg, while the concentration of 2 mg per liter of BA significantly excelled on the rest of the concentrations by giving it the highest fresh weight of 0.49 mg, and the control treatment gave the lowest fresh weight of callus of 0.04 mg.

**Keywords**: auxin, cytokinin, callus induction

**introduction**

*Nephrolepis exaltata* L. 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 improved 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 floriculture industry around the world (2).Effective laboratory culture of the plant begins with the use of optimized aseptic technique. The choice of time period and chemical agents depends on the sensitivity of the extracted plant to be sterilized (3). There are many common disinfectants for surface sterilization of plant materials. Common disinfectants are sodium hypochlorite, ethanol, mercuric chloride, calcium hypochlorite, silver nitrate, hydrogen peroxide, bromine water, and Tween 20 (4). Optimal tissue growth may vary between

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plants according to their nutritional requirements. (5). Growth regulators are among the most important factors in tissue culture added to the nutrient media to make the process of micropropagation of plants successful, and auxins and cytokines are among the most widely used regulators for this (6).Adding various artificial growth regulators to the nutritional media in tissue culture is considered one of the essential matters in order to encourage and stimulate explant to grow, develop, and form roots. Therefore, some plants in tissue culture do not succeed without the use of growth regulators (7 and 8). The use of growth regulators, including various cytokinins, as well as auxins, in nutrient media used in tissue culture to multiply the number of shoot in prolific plants, as it has been observed that the type used and its concentration affects the multiplication of branches, according to what was produced by various studies on many plants propagated in this way (9). Agriculture aims to: propagate the plant excelled, study the effect of auxins and cytokines on the Nephrolepis exaltata plant.

**Materials and methods:**

This experiment was carried out 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 nutritional medium was used (10). The parts of the *Nephrolepis exaltata* plant were placed in a laboratory beaker with a capacity of 250 ml, washed with water and liquid soap, then 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 a substance. Sodium hypochlorate NaOCI for the purpose of sterilizing explant and concentrations (0, 2, 4, 6) for (5, 10, 15, 20) minutes, then transferred to a 250 ml glass container containing 70% ethyl alcohol for a minute and then washed. With sterile distilled water three times to remove the remains of sterilizing materials, then they were placed in Petri dishes that had previously been sterilized with alcohol and burned with flame. They were 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 lux 1 (16) hours of light followed by 8 hours of darkness alternately, and the results were taken two weeks after culture.

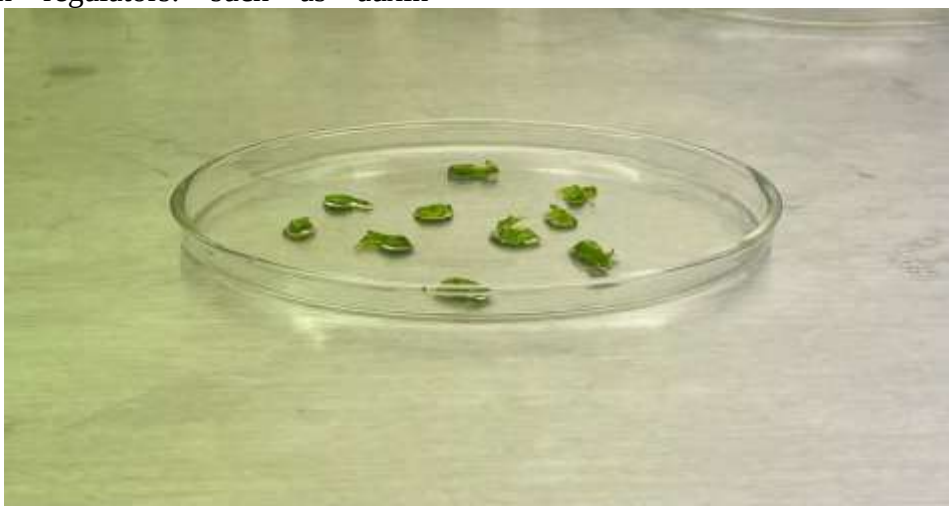

**Figure (1) Plant mowing prepared for culture**

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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 (2-4-D)-2.4 according to the requirements of the experiment. Adjust the pH 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 know the effect of 2-4-D and BA (mg L-') and their interaction in growing *Nephrolepis exaltata* explant in nutrient media. The explants were grown on sterile media (Murashige, and Skoog, 1962) provided with different concentrations of growth regulators. 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.

**Results and discussion**

**The effect of sodium hypochlorate concentrations and sterilization period on the percentage of contamination of growing shoots after 15 days of culture**

The contamination rate was 100% in the control treatment, and the contamination rate decreased with increasing sterilization duration. The contamination rate reached 63.25% at 20 minutes. On the other hand, the 4% concentration gave the lowest contamination rate, amounting to 28.25%, and the interaction effect was between The effect of sodium hypochlorate concentrations and the sterilization period in reducing the rate of contamination of explant. Sodium hypochlorate concentrations and the time period significantly decreased the contamination rate to reach 26.00% at concentration 4 by interfering with the duration of 15 minutes, and the growth of the crops was good. Inappropriate concentrations of sterilizers have a fatal effect. It affects cell division and restricts the growth and development of the explant. Therefore, the appropriate concentration, formulations, and duration of exposure to the sterilizer is essential for the success of culture in the laboratory (11). The reason for using sodium hypochlorate in surface sterilization of explant may be due to its efficiency and its lack of harm to the explant at the appropriate concentration, and that the concentration and time period are effective in reducing surface contamination of explant grown excelled, and that a concentration higher than the ideal concentration and a long period of time may lead to death. Explant: This is consistent with what researchers found (12 and 13), as they stated that high concentrations lead to the death of explant. In vitro sterilization is an essential step for plant tissue culture and the final results of in vitro culture depend directly on the efficiency of sterilization (14). Success in plant tissue culture and launching plant regeneration protocols depends greatly on the efficiency of the sterilization phase (15)(16).

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

| Concentration Average | Time | | | | Concentration |
|---|---|---|---|---|---|
| | 20 | 15 | 10 | 5 | |
| 100 | 100 | 100 | 100 | 100 | 0 |
| 82.67 | 71.00 | 78.00 | 81.67 | 100 | 2 |
| 28.25 | 30.67 | 26.00 | 27.67 | 28.67 | 4 |
| 51.25 | 51.00 | 52.00 | 50.00 | 51.67 | 6 |
| | 63.25 | 64.00 | 64.83 | 70.08 | time average |
| | 8.88=interaction | | 4.44= time | concentration 4.44= | L.S.D 0.05 |

**Effect of (2,4-D) 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 (2,4-D)D,2,4- in increasing the percentage rate of callus induction from the growing top of the *Nephrolepis exaltata* ( plant after six weeks of culture, as the concentration of 3.0 mg/L significantly excelled on all treatments by giving it the highest percentage of 53.3. % followed by 2.0 mg at that concentration. litres" (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, as the two concentrations excelled on 2 mg/L and 1.5 mg/L. liter over 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 (17 and 18).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 comparative treatment was given. Cytokinins are used in low concentrations to produce physiological effects in the cultivated explant, and in balance with auxins, they help in inducing callus (19). Many researchers have indicated the possibility of inducing callus depending on the explant. Growth regulators added to the nutrient medium and a balanced combination increase the power of callus stimulation (20))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 (21). In previous years, studies have developed in the field of callus induction and its use in the production of secondary metabolite compounds from medically important plants and comparing them with the original plant or growing in the field, as these compounds are considered to have high biological effectiveness and are highly stable compared to compounds produced artificially (22).

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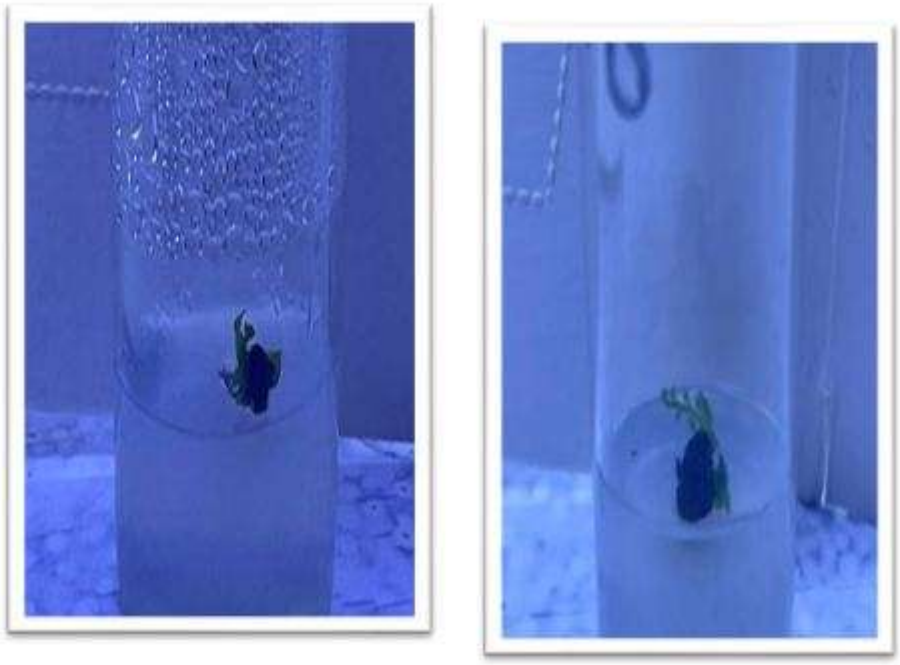
**Table (2) Effect of (2,4-D) and BA on the percentage of callus induction from the growing apex after 6 weeks of culture.**

| 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 | BA average |
| | 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 the *Nephrolepis exaltata* plant in the nutrient medium prepared with a concentration of (2,4-D)-3.0 and BA 2.0.

**Effect of BA and 2,4-D interaction between them on the fresh weight of callus induced from the growing shoot after 6 weeks of culture.**

The results in Table (3) showed that concentrations of 2,4-D had a significant effect on increasing the rate of fresh weight of callus produced from the growing shoot of the Nephrolepis exaltata plant after six weeks of culture. All concentrations of 2,4-D used were significantly excelled on controltreatment. It gave Concentration 3 mg. A liter of 2,4-D had the highest fresh weight of 0.52 mg, while the control treatment gave the lowest fresh weight of 0.01 mg. This result is consistent with (23) who used different concentrations of D-24 only to induce and develop callus induced from seeds of the Boerhaavia paniculata plant, as the seeds did not respond to callus formation in media devoid of this auxin. It also agrees with (24) who indicated that concentrations D-2.4 was ideal for inducing callus from star anise leaves. Using different concentrations of BA had a significant effect on increasing the fresh weight of the callus compared to the medium without it (control), as the concentration of 2 mg per liter of BA was significantly superior to the rest of the concentrations by giving it the highest fresh weight rate. It reached 0.49 mg, and the control treatment gave the lowest fresh weight of callus, which amounted to 0.04 mg. The

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effect of cytokinins in inducing callus may be due to their effective role in creating "sinks" in places where they accumulate to accelerate the transfer of water and nutrients, thus urging cells grown excelled on divide and grow, which leads to an increase in the weight of the callus. This happens at ideal concentrations, but at high concentrations Some of them may lead to adverse results, as they may be toxic in this case (25). The results of the same table also indicated that there was a significant effect of the interaction between the growth regulators D-2,4 and BA in increasing the fresh weight of the induced callus when the combination of 2,4-D was given at a concentration of 3 mg. L + BA at a concentration of 2 mg L was the highest concentration for the fresh weight of the callus, which was 1.61, while the control treatment did not give any fresh weight of the callus because it did not respond to callus formation. These results are consistent with what previous studies indicated that a nutrient medium devoid of growth regulators does not encourage the formation of callus from plant parts grown on it, while the ability of these parts to form callus increases when the nutrient medium is prepared with growth regulators at different concentrations and the ideal compatibility between them is reached. It is compatible with the internal content of hormones in plant parts. The best results for inducing callus and increasing its growth from various plant parts, such as Antognani (Pasiflora spp.) and Kelussia odoratissima Razeghi, were obtained when the medium was prepared with D-2,4 and Kin. (26) used the medium prepared with D-2,4 and BA to obtain the best growth of callus induced from Verbascum leaves. The reason for increasing the growth of fresh callus weight is due to adding the appropriate concentration of benzyl adenine, meaning that BA works to balance the positive and negative charges on both ends of the cell membrane and thus increases the absorption of other growth regulators, which lead to an increase in the level of biosynthesis, including increased protein synthesis and division. cellular activity, leading to an increase in fresh weight (27). The reason for increasing the fresh weight of the callus is due to the addition of auxin, which stimulates the plasticity of the cell wall by breaking the bonds of the cell wall and returning them to new locations under swelling pressure, which increases the cell size and expansion (28).

**Table (3) The effect of 2,4-D and BA and their interaction on the fresh weight of callus induced from the growing apex after 6 weeks of culture.**

| 2 ،4 – D average | BA | | | | | **2,4-D** |
|---|---|---|---|---|---|---|
| | 2 | 1.5 | 1 | 0.5 | 0 | |
| 0.01 | 0.07 | 0.00 | 0.00 | 0.00 | 0.00 | 0 |
| 0.04 | 0.11 | 0.10 | 0.00 | 0.00 | 0.00 | 1 |
| 0.25 | 0.53 | 00.34 | 0.16 | 0.13 | 0.09 | 2 |
| 0.52 | 1.61 | 0.58 | 0.18 | 0.13 | 0.10 | 3 |
| 0.04 | 0.15 | 0.09 | 0.00 | 0.00 | 0.00 | 4 |
| | 0.49 | 0.22 | 0.06 | 0.05 | 0.04 | BA average |
| | 0.174=interaction | | | 0.078= BA | 0.078=D,2.4 | L.S.D 0.05 |

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