STUDY THE EFFECT OF COCONUT WATER AND SOME GROWTH REGULATORS ON ORCHID PROPAGATION *IN VITRO*

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ABSTRACT

The study was conducted in the Central Laboratory of Plant Tissue Culture - College of Agricultural Engineering Sciences - University of Baghdad for the period from October 2021 to August 2022 with the aim of tissue propagation of the *Phalaenopsis amabilis* L. orchid plant. The study consisted of five sequential experiments (sterilization, initiation, multiplication, rooting, and acclimatization) in order to obtain the best treatment for the next experiment. The results showed that the best treatment for sterilizing the nodules was 15 ml of commercial bleach for 15 minutes. As for the Initiation stage, the nodules were stimulated on MS medium containing 60 ml L^{-1} coconut water, which amounted to 100%. As for the multiplication stage, the interaction between BA and NAA showed a significant effect on the average number of shoots, their length, and the number of leaves, where the concentration of 2.5 mg L^{-1} BA in interaction with 1 mg L^{-1} of NAA produced the highest rate of shoots number (4 shoots. explants^{-1}), leaves number.shoot^{-1} (4 shoot^{-1}). The best medium for rooting the shoots was using MS medium half the strength of salts prepared with IBA at a concentration of 1 mg L^{-1} Where it produced the highest rate of rooting (100%), number of roots (9 root shoot $^{-1}$) and root length (6 cm.). As for acclimatization, the highest surviving rate (90%) was obtained in the mediums: river soil, peat moss, Perlite and tree bark, peat moss, perlite at a ratio of 1:1:1 for each.

Keywords: commercial bleach, single nodes, shoots, peat moss , bark
Part of Thesis for the 1st author

دراسة تأثير ماء جوز الهند وبعض منظمات النمو في اكثار نبات الأوركيد خارج الجسم الحي

ديمة غازي علي الغانمي لمياء خليفة جواد العامري
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المستخلص

نُفذت الدراسة في مختبر زراعة الانسجة النباتية المركزي – كليه علوم الهندسة الزراعية –جامعة بغداد للمدة من شهر تشرين الأول 2021 حتى اب 2022 بهدف اكثار نبات الأوركيد *Phalaenopsis amabilis L.* نسيجيا.اشتملت الدراسة على خمس تجارب متسلسلة وهي :التعقيم ,النشوء,التضاعف ,التجذير والأقلمة بهدف الحصول على افضل توليفة للمرحلة اللاحقة لكل تجربة . اظهرت النتائج ان افضل معاملة لتعقيم العقد هي 15 مل من القاصر التجاري لمده 15 دقيقه و التي لم تعطِ اي نسبة تلوث اما مرحلة النشوء فقد تحفزت العقد واعطت اعلى نسبة استجابة على وسط MS حاوي على 60 مل لتر$^{-1}$ ماء جوز الهند بلغت 100% , وبالنسبة لمرحلة التضاعف فقد اظهرت معاملة التداخل بين BA و NAA تاثيراً معنوياً في معدل عدد الافرع واطوالها وعدد الأوراق ,اذ أعطى التركيز 2.5 ملغم لتر$^{-1}$ BA بالتداخل مع 1 ملغم لتر$^{-1}$ من NAA أعلى معدل لعدد الافرع بلغ 4 فرع جزء نباتي$^{-1}$ واعلى معدل لعدد الأوراق بلغ 4 ورقه فرع $^{-1}$,اما صفة الطول فقد اعطت معاملة المقارنة والتركيز1.5ملغم لتر$^{-1}$ BA مع 0 ملغم لتر$^{-1}$ NAA اعلى معدل بلغ 3 سم و الذي اختلف معنويا مع اقل قيمة بلغت1سم عند التركيز 2.5 و3.5 ملغم لتر$^{-1}$ مع 0 ملغم لتر$^{-1}$ NAA, وان افضل وسط لتجذير الافرع كان باستخدام وسط MS نصف قوة املاح المجهز ب IBA بتركيز 1 ملغم لتر$^{-1}$ حيث أعطى أعلى نسبه تجذير 100% واعلى معدل لعدد الجذور بلغ 9 جذرفرع $^{-1}$ واعلى معدل لطول الجذر بلغ 6 سم وتم الأستفادة من نتائج التجارب السابقة جميعها لغرض ادخالها في المرحلة النهائية (الاقلمة) اذ تم الحصول على اعلى نسبه بقاء بلغت 90 % عند الوسطين زميج, بيتموس ,برلايت و قلف, بيتموس, برلايت وبنسبة 1:1:1 لكل منهما.

الكلمات المفتاحية: القاصر التجاري, العقد المفرده, الافرع, البيتموس, القلف
مستل من رسالة ماجستير للباحث الاول

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INTRODUCTION

The orchid plant belongs to the Orchidaceae family, which includes 736-889 genera, 27,800 species, and more than 100,000 hybrids worldwide (3, 13) It includes onocotyledonous perennial herbs, the nature of its growth differs in different races. 85% of the species grow in areas close to the equator and between the Tropics of Capricorn and Cancer, and this means that they are spread in tropical and subtropical regions. Orchids grow in two forms, the first as epiphytic plants, where they grow on the shoot and stems of trees, and sometimes on rocks .As for the other picture, it represents terrestrial plants, and this group grows in the soil, where it obtains its food, like other plants, as in the genus Calanthe (24).Orchids occupy a distinguished position in the trade of cut flowers in the world, and they are among the most beautiful and expensive cut flowers due to their longevity on the plant as well as their longevity in the vase, which reaches about a month (9).Orchids reproduce sexually by very small seeds, and one gram contains three million seeds. The germination of orchid seeds depends on the presence of certain fungi that live with seedlings in a symbiotic way. The fungi threads grow between the growth environment and plant tissues to supply them with nutrients. This symbiotic life is necessary for orchids, especially in the early stages of growth. In order for the plants produced from the seeds to reach the size of the flowers, it takes several years, ranging between 5-7 years. It also reproduces by dividing or by successions for some species, or by non-true bulbs (pseudo bulbs) (23) However, its culture in the field requires an area of land, service operations, and a relatively long time until the plant is fully matured, in addition to the risks of field culture represented in the uncertain climatic and environmental conditions, which negatively affect the growth and yield of these plants, and then the quantity and quality of active substances, and this prompted some researchers to use technology Tissue culture in the propagation of orchids because of its ability to produce large numbers of plants similar to the mother plant in a small area and a relatively short time and throughout the year (16, 18, 21) The growth, multiplication and rooting of the shoots *invitro* is affected by several factors, including growth stimuli such as cytokinins and auxins, as well as some added extracts such as coconut water (28).Based on the foregoing, the research aims to: Tissue propagation of orchids by determining the combination of growth regulators for all stages of propagation. * Studying the effect of coconut water on the response and Initiation of explant.

MATERIALS AND METHODS

The study was conducted in the Central Laboratory for Plant Tissue Culture - College of Agricultural Engineering Sciences - University of Baghdad. The seedlings were taken from one of the private nurseries.The prepared medium MS (27), produced by the Dutch company HIMEDIA, was used with a weight of 4.9 $g.L^{-1}$ in all stages except for the experiments in which half of the MS was used, where it was added with a weight of 2.45 $g\ L^{-1}$ (10)Sucrose was added in an amount of 30 gL^{-1} (5), and plant growth regulators were added after they were prepared as base solutions according to the type of experiment, then the pH (Potenz Hydrogen) was adjusted to 5.7 ± 0.1, using HCl Hydrochloric acid or sodium hydroxide NaOH Sodium hydroxide one standard, then complete the volume to one liter and add an agar of the type (Agar-Agar) in an amount of 7 $g\ L^{-1}$ to the medium, and for the purpose of homogenizing the components and dissolving the agars, preheat the food medium using the hot plate magnetic stirrer until homogeneity and distribute Then in culture tubes with an amount of 10 ml and covered with appropriate caps. All used tools, including blade holders, tweezers, and petri dishes, were sterilized using the OVEN device at a temperature of 160°C for 2 hours. In addition to using ethyl alcohol at a concentration of 99% to sterilize the tweezers and blades and burn them with a Bunsen lamp after each implantation process inside the Laminar air flow cabinet. The planting booth and hands were sterilized with 70% ethyl alcohol. As for the distilled water used in agriculture, it was sterilized using an autoclave device at a temperature of 121 ° C and under a pressure of 1.04 kg cm-2 for 30 minutes, then left to cool until it is ready for use. The tubes containing the medium were sterilized with an

autoclave device at a temperature of 121 ° C and under a pressure of 1.04 kg cm-2 for 15 minutes, then the medium was left to cool and harden at room temperature until it was ready for culture. The explants were cut with a length of one node and left under running tap water for 30 Minutes, after which they were washed with liquid soap and then with distilled water. Then it was transferred to the laminar airflow cabin, where it was sterilized by commercial bleach, with different concentrations (0, 5, 10, 15) ml per 100 ml, and for different periods of time (10, 15, 5) minutes (6). With the addition of a drop of tween-20, and after ten days, measurements were taken that represented the percentage of contamination (12).

Pollution percentage = number of polluted plants/total number * 100

After determining the best result obtained from the sterilization experiment, the plant parts were sterilized and cultivated according to the follow in experiments:

1. Effect of MS medium strength and BA concentrations and the interaction between them on the Initiation of shoots:Plant parts (single nodes) were cultured on MS medium at full and half strength, supplemented with different concentrations of BA (1, 0, 2, 1.5) mg L^{-1}.

2. Effect of BA and NAA on the Initiation of shoots**:** Explants (single nodes) were grown on MS medium supplemented with different concentrations of BA (1.5, 1, 0, 2) mg L^{-1}, by interaction with NAA at concentrations (0.3, 0.2, 0.1, 0) mg L^{-1} (35)

3. Effect of BA and coconut water on the Initiation of shoots: Explants (single nodes) were cultured on MS medium supplemented with concentrations of BA at concentrations of (0, 1, 1.5,2) mg L^{-1} in interaction with coconut water and at concentrations (0, 30, 60, 90) ml L^{-1}. (36) After four weeks, the results were obtained, represented by the percentage of response to the plant parts and the three experiments. Response rate = number of stimulated plants / total number * 100

The vegetative growths resulting from the Initiation stage were cultured on MS medium supplied with different concentrations of BA 0, 2.5, 1.5, 3.5 (mg L^{-1}) by interaction with NAA at concentrations (0, 5, 1, 1.5) mg L^{-1} (11).after 6 weeks of culture, the results represented by the number of shoots, their lengths and the number of leaves were obtained.after that the resulting roots were taken from the best treatment of the multiplication stage and planted on MS medium with full strength, half strength and a quarter strength, with different concentrations of IBA acid added (0, 1.5, 1, 0.5) mg L^{-1} added to it 30 g L^{-1} sugar (41). Then the results were taken after 6 weeks of culture, represented by the percentage of rooting, as follows:

Rooting percentage = number of rooted shoots / total number x 100

The crops were incubated in the growth chamber under a light intensity of 1000 lux for 16 hours of light and 8 hours of darkness at a temperature of 25 ± 2 .The rooted shoots were extracted from the test tubes and their root system was washed with running water to get rid of the remnants of the agar, then immersed in a solution containing half strength of MS salts for the purpose of hardening and preparing the plants for transfer to the soil and left for a week in the growth room (36). After that, several mediums consisting of peat moss, perlite, bark and peat moss, perlite, zigzag, peat moss and perlite were prepared, then sterilized in an autoclave for 30 minutes at a temperature of 121 °C and a pressure of 1.04 kg cm^{-2}. The roots of the plants were treated with the fungicide Pentanol at a concentration of 1 ml L^{-1}.Then it was culture in plastic pots with a diameter of 5 cm. After that, it was watered and covered with a plastic cover to maintain moisture, after which the cover was pierced, then the number of holes increased until the cover was gradually lifted after 4 weeks, then it was transferred to the plastic house after 6 weeks. All experiments were analyzed using a completely randomized design (CRD) with one or two factors, with 10 replicates for each treatment. The results were analyzed using (38) in analyzing the data to study the effect of different factors and their interactions on the studied traits, and the significant differences between the averages were compared with the Least Significant Difference-LSD test. In addition to the t-test at the 5% probability level (7).

RESULTS AND DISCUSSION

Initiation stage:

Effect of commercial bleach on the percentage of contamination of explants (single node) of orchids: The results in Table (1) show the effect of different concentrations of the commercial bleach, which had a significant effect on reducing the percentage of contamination, where the percentage of pollution reached 33.33% at a concentration of 15 ml, which differed significantly from the rest of the treatments. While the control treatment gave the highest percentage of contamination amounted to 100%.The results in the same table show that the sterilization periods have a significant effect in reducing pollution rates if the 15th period gave the lowest pollution rate of 55%, compared to the 5-minute period, which gave the highest pollution rate of 85%, which differed significantly from the 10-minute period. As for the bilateral interaction between concentrations and sterilization periods, the results of the table showed that the interaction between concentration 15 and 15 minutes was significantly excelled on the rest of the treatments, which gave the lowest contamination rate of 0%. We note that the pollution decreases with the increase of the minor, up to the stage of eliminating pollution at a concentration of 15%, due to the effectiveness of hypochlorous acid (HOCI), which is a strong oxidant, and is formed as a result of the combination of chlorine with water (8).This is consistent with what Ramawat (33) concluded that NaOCl is an effective substance in sterilizing plant tissues, where it leads to the death of pathogens.

$Cl2 + H2O \rightarrow HCl + HOCl$

These results are consistent with the results of Balilashaki and others (12) when sterilizing the knots of the orchid plant genus Phalaensis, where it was found that a concentration of 7% of NaOCl gave the lowest percentage of contamination, as studies indicate that sodium hypochlorite has a high efficiency in sterilizing the knots of the orchid plant.

Table 1. Effect of concentrations of commercial bleach and durations of immersion and the interaction between them on the percentage of contamination of explants (single node)

average	Duration (minutes)			concentration ml/ml 100
	15	10	5	
100	100	100	100	0
90.00	80	90	100	5
53.33	40	50	70	10
33.33	0	30	70	15
---	55	67.50	85.00	average
: Duration ، * 18.23 :concentration :LSD values * 31.57 : interaction ، * 15.79 .(P≤0.05) *				

The effect of BA concentrations and the strength of the MS medium and the interaction between them on the response rate of explants (single node):

The results in Table (2) show that the strength of the MS medium has a significant effect on the response rate of single nodes, as it gave the highest response rate on the MS medium with full strength, reaching 32.50%, which did not differ significantly with the MS half treatment, which gave 12.50%. As for the concentrations of BA, the concentration 1.5 mg L^{-1} was significantly excelled on the rest of the concentrations, as it gave the highest percentage of 45%, and did not differ significantly from the concentration 1 mg $L^{-1,}$ which gave 25%. BA the interaction treatment between full strength MS with 1.5 mg L^{-1} of BA gave the highest response rate of 60%, which differed significantly with the rest of the treatments, except for the interaction treatment between full strength MS with 1 mg L^{-1} BA, which gave a response rate of 40%, and the half-strength MS treatment. With 1.5 mg L^{-1} BA, which gave 30%.

Table 2. Effect of MS medium strength and BA concentrations and the interaction between them on the response rate of cultivated explants (single nodes)

average	BA concentrations(mg)				MS strength
	2	1.5	1	0	
12.50	10	30	10	0	2/1strength
32.50	20	60	40	10	full strength
---	15.00	45.00	25.00	5.00	average
values LSD :MS ، * 17.58 :BA ، * 24.86 : interaction* 35.16 : *P≤0.05					

The effect of BA and NAA concentrations and the interaction between them on the response rate of explants (single nodes)

The results in Table (3) showed that BA had a significant effect on the response of single nodes .As the concentration of 1.5 mg L^{-1} of BA gave the highest response rate of 72.50%, which did not differ significantly with the concentration of 1 mg L^{-1}, which gave 57.5%, while it differed significantly with the rest of the treatments. As for NAA concentrations, the treatment of 0.3 mg L^{-1} gave the highest response rate of 50%, which did not differ significantly with the rest of the treatments. As for the bi-interaction between BA and NAA, it had a significant effect. The two interaction treatments gave 1.5 mg L-BA with 0.2 and 0.3 mg L-NAA the highest response rate of 80%, which differed significantly with the control treatment. While the addition of NAA concentrations alone did not give any response. The rate of stimulation and growth of buds on the cultivated explants are affected by the concentration and quality of cytokinins and auxins added to the nutrient medium. The higher the concentration of cytokinin / auxin, the Initiation of lateral and transverse shoots is stimulated, while the roots are stimulated when the concentration of auxin / cytokinin is high. (16).

Table 3. Effect of BA concentrations and NAA concentrations and the interaction between them on the response rate of explants (single nodes) grown on MS medium

average	NAA conc.(mg)				BA conc. (mg)
	0.3	0.2	0.1	0	
2.50	0	0	0	10	0
57.5	70	60	60	40	1
72.5	80	80	70	60	1.5
32.5	50	30	30	20	2
---	50.0	42.50	40.00	32.50	average
values LSD :BA ، * 9.37 :NAA ، * 9.37 : interaction. * 15.82 : *P≤0.05					

The effect of BA and coconut water and the interaction between them on the response rate of explants(single node)

The results of the statistical data in Table 4 show the excelled of the BA-free food medium containing 1.5 mg L^{-1} in giving them the highest response rate of 62.50% for each of them, which differed significantly with the concentration of 2 mg L^{-1}, which gave the lowest response rate of 30.0%.The concentrations of CW also had a significant effect, as the concentration of 60 ml L^{-1} gave the highest rate of response for single nodes, which amounted to 75%, while the control treatment gave the lowest response rate of 32.5%. As for the interaction treatments between BA and CW, the treatment of 60 ml L^{-1} of CW was excelled with 0 mg.L^{-1} BA significant. As it gave the highest rate of response to nodes reached 100%, which did not differ significantly with the treatment of 60 ml L^{-1} and 1 mg L^{-1} BA compared with the control treatment that gave 10%.The response rate increased with the increase in the concentration of coconut water, reaching the best concentration of 60 ml.L^{-1} after which the percentage began to decrease, and the reason for this could be due to its auxin content known to show apical dominance in the plant or the toxic effect of cytokinins as a result of high concentrations. The effect of coconut water on the response rate of a single nodule may be due to the presence of cytokinins in it such as (Zeatin Glucosid) Zeatinriboside, Zeatin which is known to prevent or reduce apical dominance, which encourages the growth of lateral buds. It should be noted that cytokinins are not found alone but in combination with other plant hormones such as auxins and gibberellins, chemical compounds that may exert synergistic effects with the cytokinins (Wu and Hu, 2009). This is consistent with what was found by Kelapa et al. (2013) which showed that coconut water contains auxins (IAA) and cytokinins that gave good results for stimulating bud growth.

Table 4. Effect of BA concentrations and C.W concentrations and the interaction between them on the percentage response of explants (single nodes) grown on MS medium

average	C.W(ml) conc.				BA conc. (mg)
	90	60	30	0	
62.5	80	100	60	10	0
60.0	60	90	50	40	1
62.5	60	70	60	60	1.5
30.0	20	40	40	20	2
---	55.00	75.00	52.50	32.50	average
، * 11.02 :C.W ، * 11.02 :BA :LSD values . * 19.42 : interaction .(P≤0.05) *					

multiplication stage:

The effect of BA and NAA and the interaction between them on the average number of shoots: The results of Table (5) showed that BA concentrations had a significant effect on increasing the number of shoots. The concentration of 2.5 mg L^{-1} was excelled by giving it the highest rate of 2.50 shoots explant^{-1}, which did not differ significantly from the concentration of 1.5 and 3.5 mg L^{-1} compared with the control treatment that gave the lowest average of the number of shoots amounted to 1 shoot .explant^{-1}.As for the effect of NAA, the results of the table indicate that there are no significant differences between the concentrations. However, the concentration of 1 mg L^{-1} gave the highest rate of the number of shoots, which amounted to 2.25 shoots .explant^{-1}, compared with the control treatment, which gave the lowest number of shoots, which amounted to 1 shoot .explant^{-1} .As for the interaction between the concentrations of BA and NAA, the results in the same table indicate that the coefficient of interaction between the concentration of 2.5 mg L^{-1} of BA with 1 mg L^{-1} of NAA has been significantly excelled by giving it the highest rate of the number of shoots of 4 shoots, explants^{-1}, compared with the control treatment that gave 1 shoot .explant^{-1}. The reason for the increase in the number of shoots is due to the role played by cytokinins at appropriate concentrations in tissue culture in terms of their action in breaking the apical dominance and creating attractions in the lateral buds, and this stimulates the speed of nutrients transfer to them, which results in stimulating the growth of buds (2) and (14). As for the reason for obtaining the highest rate of the number of shoots when treating the interaction between the concentration of BA and NAA, it may be due to the fact that the effectiveness of cytokinins in causing replication increases in the presence of auxin, i.e. the effect increases when they are present together in the food medium (26, 29).These results agreed with what was found by Pant and Thapa (32) when Dendrobium primulinum Lindl was propagated by adding (NAA) and benzylaminopurine (BAP) to medium.

Table 5. The effect of BA and NAA and the interaction between them on the average number of shoots of orchids (shoot.explant-1) after 6 weeks of culture on MS medium.

average	NAA conc. (mg) 1.5	1	0.5	0	BA conc. (mg)
1.0	1	1	1	1	0
1.5	1	2	2	1	1.5
2.5	2	4	3	1	2.5
1.5	1	2	2	1	3.5
---	1.25	2.25	2.0	1.0	average
values LSD :BA ، * 1.43 :NAA 1.43 :NS ، interaction) *.* 2.27 : (P≤0.0).					

The effect of BA and NAA and the interaction between them on the average length of multiplied shoots

The results of Table (6) show the effect of different concentrations of BA and NAA on the average shoot length. With regard to the effect of BA concentrations, the results showed that the control treatment was significantly excelled.It gave the highest mean for the length of the shoots amounted to 2.33 cm, compared to the lowest mean for the concentration of 2.5 mg L^{-1}, which amounted to 1.55 cm, which differed significantly with it. As for the concentrations of NAA, the control treatment excelled by giving it the highest mean for the length of the shoots amounted to 2.00 cm, which did not differ significantly with the rest concentrations. As for the effect of interference between NAA and BA The results in the same table indicate the excelled of the control treatment and the concentration treatment of 1.5 mg L^{-1} of BA with 0 mg L^{-1} of NAA significantly by giving them the highest average shoot length of 3 cm, while the treatment recorded 2.5 and 3.5 mg L^{-1} of BA and 0 mg L^{-1} of BA NAA The lowest average shoot length was 1 cm.

Table 6. The effect of BA and NAA and the interaction between them on the average shoot length of orchids (cm) after 6 weeks of culture on MS medium.

average	NAA conc. (mg) 1.5	1	0.5	0	BA conc. (mg)
2.33	2.1	2	2.2	3	0
1.87	1.8	1.5	1.2	3	1.5
1.55	1.7	1.5	2	1	2.5
1.77	2.3	1.8	2	1	3.5
---	1.97	1.70	1.85	2.00	average
values LSD :BA ، * 0.681 :NAA 0.681 :NS ، interaction) *. * 1.208 : (P≤0.05).					

The effect of BA and NAA and the interaction between them on the average leaf number of multiplied shoots

The statistical results show in Table 7) that the concentration 2.5 mg liter BA $^{1-}$ excelled as it gave the highest average number of leaves of 2.85 leaves, shoot $^{1-}$, while the control treatment gave the lowest average number of leaves, amounting to 1.12 leaves, shoot $^{1-}$.As for NAA, the concentration of 1 mg L^{-1} was significantly higher by giving it the highest average number of leaves, which reached 2.57 leaves, shoot$^{-1.}$As for the effect of interaction, the treatment of bilateral interaction between BA and NAA gave the highest rate of leaf number, which reached 4 leaf shoot^{-1} at concentrations of 2.5 mg L^{-1} of BA and 1 mg L^{-1} of NAA. The reason for the increase in the number of leaves when adding BA may be due to the cytokinins that play an important role in increasing the number of leaves by encouraging vegetative growth, as a result of stimulating cell division and differentiation and attracting nutrients to the explant treated with it (20).

Table 7. The effect of BA and NAA and the interaction between them on the average number of orchid leaves (shoot leaf^{-1}) after 6 weeks of culture on MS medium.

average	NAA conc. (mg) 1.5	1	0.5	0	BA conc.(mg)
1.12	1	1.5	1	1	0
1.27	1	1.8	1.3	1	1.5
2.85	2.9	4	2.5	2	2.5
2.25	2.5	3	2	1.5	3.5
---	1.85	2.57	1.70	1.37	average
interaction ، * 1.13 :NAA ، * 1.13 :BA :LSD values * 2.06 : (P≤0.05) *					

rooting stage

Effect of MS strength and IBA concentrations and the interaction between them on the percentage of rooting

The results in Table (8) indicate that the addition of IBA to the rooting medium increased the rate of rooting.The concentration of 1 mg L^{-1} gave the highest average of 66.67%, which was significantly excelled on the concentration of 0.5, which gave a rooting rate of 23.33%. While the control treatment failed to form roots. As for the effect of salt strength, it was found that the use of 0.5 MS raised the rooting percentage to 52.50%, which differed significantly with the rest of the treatments. As for the effect of interaction between IBA with MS salts, the concentration of 1 mg L^{-1} of IBA with 0.5 MS gave the highest rooting rate of 100%.The concentration of 0.5 mg L^{-1} of IBA with 0.25 MS gave the least rooting rate of 10%, while the control treatment (0 BA with all MS powers) did not give any rooting rate.

Table (8) Effect of MS salt strength and IBA concentrations and the interaction between them on the percentage of rooting of orchids after 6 weeks of culture

average	MS strength 1	0.5	0.25	IBA conc. (mg)
0.00	0	0	0	0
23.33	20	40	10	0.5
66.67	70	100	30	1
50.00	50	70	30	1.5
---	35.00	52.50	17.50	average
، * 15.02 :MS ، * 17.56 :IBA :LSD values * 29.85 : interaction (P≤0.05) *				

The effect of strength of MS salts and IBA concentrations and the interaction between them on the number of roots of orchid shoots

The results in Table (9) show that the use of IBA at a concentration of 1 mg L^{-1} increased the number of roots to 5.67 root shoot-1, which differed significantly from the two concentrations of 0.5 and 1.5 mg L^{-1}, which gave 1.67 and 3.67 root .shoot^{-1}, respectively. As for a control treatment, there were no roots. As for the effect of the strength of MS salts, the same table shows that half the strength of the MS salts gave the highest rate of the number of roots, which reached 4.25 root shoot $^{-1}$, which did not differ significantly with the full strength of MS, while it differed significantly with 0.25 MS strength, which gave 1.50 shoot.root $^{-1}$,The table shows that the interaction between the concentration of 1 mg L^{-1} IBA with half the strength of MS salts gave the highest average number of roots, reaching 9 shoot roots $^{-1}$, which did not differ significantly with the interaction treatment between 1.5 mg L^{-1} with half the strength of MS, which gave 6 free roots. $^{-1}$, while it differed significantly with the rest of the treatments.

Table 9. the effect of the strength of MS salts and IBA concentrations and the interaction between them on the number of roots of orchid shoots after 6 weeks of culture(shoot.root $^{-1}$)

average	MS strength			IBA Conc. (mg)
	1	0.5	0.25	
0.00	0	0	0	0
1.67	2	2	1	0.5
5.67	5	9	3	1
3.67	3	6	2	1.5
---	2.50	4.25	1.50	average
، * 1.87 :MS ، * 1.87 :IBA :LSD values * 3.19 : interaction (P≤0.05) *				

The effect of strength of MS salts and IBA concentrations and the interaction between them on the average root length of orchid shoots

The results in Table 10 indicate that the use of a concentration of 1 mg L^{-1} of IBA led to an increase in the root length of 4 cm, while the concentration of 1.5 mg L^{-1} of IBA gave the least root length of 2.00 cm.As for the strength of MS salts, the 0.5 MS treatment was significantly excelled on the rest of the treatments by giving it the highest average root length of 3.63 cm.As for the interaction, the interaction between IBA at a concentration of 1 mg L^{-1} with half the strength of MS salts gave the highest average length of 6 cm and did not differ significantly from the concentration 0.5 mg L^{-1} IBA with half the strength of MS. It gave a root length of 5.5 cm, and it did not differ with the interaction treatment between a concentration of 1 mg L-1 and 0.25 of MS, which gave 4 cm, while it differed significantly with the rest of the treatments compared with the control treatment that failed to form roots.

Table 10. Effect of MS salts strength and IBA concentrations and the interaction between them on root length of orchid shoot after 6 weeks of culture(cm)

average	MS strength			IBA CONC. (mg)
	1	0.5	0.25	
0.00	0	0	0	0
3.40	3	5.5	2	0.5
4.00	2	6	4	1
2.00	2	3	2	1.5
---	1.75	3.63	2.00	average
، * 1.57 :MS ، * 1.82 :IBA :LSD values . * 2.93 : interaction .(P≤0.05) *				

Hartmann et al., (20) reported that auxins are known to increase rooting percentage and reduce rooting period, in addition to regular rooting. IBA is a form of auxin that is effective in rooting a large number of plant species. Research evidence also indicates that auxins play a major role in determining rooting efficiency (17). The positive effect of auxins appears based on its important role in activating cell division of the cambium-generating layer, thus leading to increased root formation and the formation of root initiators (20 and 31). The presence of auxins is important in stimulating the growths to rooting and works on stimulating and elongating the roots. In general, auxins work to increase the rate of ion exchange, enzymatic activity, cellular permeability, and the rate of transmission of signals that increase the rate of root formation of the cultivated shoots, due to its important and direct role in the rooting stage (22). The concentration of internal or added auxin affects the division of root cells. As auxin plays a major role in stimulating the Initiation of adventitious roots through its physiological effect in losing the differentiation of the specialized parenchyma cells and returning them to meristematic cells through the process of dedifferentiation and divided into the root initial component that grows and develops to be the beginnings of the roots that grow as the adventitious root component. (19),This agrees with what was obtained by Nieves and Aspuria (30), Saini et al. (34) and Abbas (1). The increase in the rooting percentage and the number of roots (Tables 8) and (9) on medium half the strength of MS salts It may be due to an increase in the ratio of carbohydrates to nitrogen (N: C) in the medium, which leads to a decrease in the nitrogen percentage in the shoots and thus an increase in the rooting ratio and an increase in the number and length of roots (4) and this is consistent with what was found by Dewir et al. (15). The reason for the increase in the length of the roots when the concentration of half the strength of the salts is due to the phenomenon of tropism of the roots as a result of the decrease in the concentration of salts by half compared to the full concentration (25).

acclimatization stage

The acclimatization process was carried out on the rooted shoots, where the plants were planted in plastic pots with different proportions of peat moss, peat moss, perlite and peat moss, perlite, tree bark 1:1:1 and perlite and peat moss in a ratio of 1:1. The results of Table 11 show that the best survival rate is The plants grown on river soil, perlite, peat moss and tree bark, perlite, peat moss, the success rate was 90% for both media. This can be explained by the fact that river soil is a good medium for the growth and spread of roots and prevents their suffocation because it has good pores for ventilation and spread, and peat moss is a good medium for providing the nutrients necessary for growth and helps to retain the moisture necessary for growth, and the bark provides good drainage and ventilation for the roots, and this is consistent with what Xiao and Zhang (40) found.

Table 11. Survival percentage of acclimatized plants using different medium of peat moss, peat moss, perlite and tree bark after 6 weeks of acclimatization

survival percentage	medium components
%90	river soil, peat moss, Perlite 1:1:1
%90	tree bark, peat moss, perlite 1:1:1
%80	peat moss, Perlite 1:1
* 7.916	L.S.D
.(P≤0.05) *	

CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

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The authors declare that they have not received a fund.

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