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EFFECT OF PLANT GROWTH REGULATORS AND EXPLANTS ON THE MICROPROPAGATION OF CAPPARIS SPINOSA L.

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Abstract

This research sought to propagate caper plants in vitro through the cultivation of explants taken from seedlings derived from in vitro tissue culture and their subsequent cultivation in the Murashige and Skoog (MS) medium.
The results show that at the initiation stage, the best percentage of dead explants was at a concentration of 1.5 mg L^{-1} when the shoot tip was treated with benzyladenine, reaching 5%. In the same treatment, the highest percentage of growth-responsive explants reached 80%. The results at the multiplication stage show that shoot tips treated with the regulator BA at a concentration of 1.5 mg L^{-1} may be superior in terms of the number of leaves, branch length, and number of branches, reaching 7.30 leaf plantlets^{-1}, 3.78 cm, and 3.50 branch plantlets^{-1}, respectively.
The results further demonstrate a statistically significant impact of kinetin on the multiplication of the node in the studied traits at a concentration of 2 mg L^{-1}, recording 6.80 leaf plantlets^{-1}, 3.95 cm, and 2.20 branch plantlets^{-1}. As for the rooting stage, of the tested concentrations, 0.5 mg L^{-1} IBA resulted in the highest rooting percentage, reaching 80% in a medium supplemented with half of the salt concentrations. The concentration of 1 mg L^{-1} achieved the highest average for the root length trait at 3.70 cm, and an average root number of 2.91 per plantlet^{-1}.

Keywords: Capparis spinosa, In vitro, Benzyladenine, Kinetin.

تأثير منظمات النمو النباتية والجزء النباتي على الاكثار الدقيق لنبات Capparis spinosa L.

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الخلاصة

نفذت التجربة لإكثار نبات القبار خارج الجسم الحي من خلال زراعة الأجزاء النباتية المأخوذة من بادرات ناتجة من الزراعة النسيجية وزراعتها في الوسط Murashige وSkoog (MS). بينت النتائج في مرحلة النشوء أن أفضل نسبة للأجزاء النباتية الميتة كانت عند التركيز 1.5 ملغم لتر$^{-1}$ عند معاملة القمة النامية بالبنزيل أدنين بلغت 5.0%، فيما أعطت ذات المعاملة أعلى نسبة للأجزاء النباتية المستجيبة للنمو وبلغت 80.0%. وأظهرت النتائج في مرحلة التضاعف ان القمة النامية بالمنظم BA قد تفوق عند المعاملة 1.5 ملغم لتر$^{-1}$ في صفة عدد الأوراق وطول الفرع وعدد الأفرع وبلغت على التوالي 7.30 ورقة نبيت$^{-1}$ و3.78 سم و3.50 فرع نبيت$^{-1}$. فكما أشارت النتائج الى وجود التأثير المعنوي للكاينتين في تضاعف العقدة في الصفات المدروسة عند المعاملة بالتركيز 2 ملغم لتر$^{-1}$ والتي سجلت 6.80 ورقة نبيت$^{-1}$ و3.95 سم و2.20 فرع نبيت$^{-1}$. أما في مرحلة التجذير فبينت النتائج أن المعاملة بالتركيز 0.5 ملغم لتر$^{-1}$ IBA أعطى أفضل نسبة تجذير وبلغ 80.0% في وسط مزود بنصف تركيز الأملاح، فيما حقق التركيز 1 ملغم لتر$^{-1}$ أعلى متوسط لصفة طول الجذر وبلغ 3.70 سم ومتوسط عدد الجذور وبلغ 2.91 جذر نبيت$^{-1}$.

كلمات مفتاحية: نبات القبار، خارج الجسم الحي، بنزل ادنين، كاينتين.

Introduction

Caper (*Capparis spinosa* L.) is a documented medicinal species indigenous to the Mediterranean region and Iraq. The shrub can tolerate high temperatures and has petiolate, leathery leaves with entire margins and white flowers tinged with pink (19). It belongs to the Capparaceae family and grows wild or can be cultivated, as in Turkey, Morocco, and many other parts of the world (8). The caper plants have dense branches that spread towards the ground, and have dark yellow and green spines and rounded or ovate leaves. The flowers are full and fragrant and consist of four sepals, each containing a distinct anther, four white to pinkish-white petals, and several long dark purple petals.

The caper plants thrive and bloom during the summer months, ceasing when temperatures drop below freezing, when its leaves drop off (1). Capparis spinosa, commonly called caper, is a common species in the genus Capparis. It possesses

significant medical, ecological and economic importance. Due to its high content, all its components are used in various medical applications, including the curing of sunburn, kidney diseases, infected wounds and arthritis. C. spinosa is also used as a spice in some foods. For this reason, studies have been carried out to enhance and promote cultivation (14). It is used in treating rheumatism, digestive problems, kidney and liver diseases, headaches, and toothache (18), as well as in treating menopausal symptoms and colon cancer. It is also considered an appetite stimulant, tonic, astringent, anti-diarrheal, helpful for spleen diseases, expectorant, and in treating paralysis, coughs, stomach disorders, and asthma. Additionally, it contains vitamins, proteins, and fatty acids (9). These compounds endow Caper with good nutritional value for humans.

In recent decades, applied studies have focused on using tissue culture technology to propagate various plants, as it allows for the production of large numbers of plants in a relatively short time and throughout the year, in small spaces. Additionally, tissue culture technology enables the stimulation and production of active compounds in large quantities for medicinal purposes (12). (23) conducted a study on the vegetative propagation of caper plants in vitro using cytokinins, such as BA. Despite the mentioned medical and nutritional importance of caper, the plant species faces extinction due to a significant neglect in its propagation process (19), weakness in seed germination (22), and a low rooting percentage which does not exceed 50% (20). Therefore, this study aims to develop a protocol for the propagation and dissemination of the plant and to understand the effect of growth regulators (cytokinins) on the development of the apical meristem and the single node of caper plants.

Materials and Methods

Experiments were conducted at the Plant Tissue Culture Laboratory of the Department of Horticulture and Landscape Engineering at Anbar University from June to January 2023 to determine the effects of different concentrations of cytokinin (BA and Kin) on the emergence and multiplication of tissue cultures of capers and the effect of the growth regulator auxin (IBA) on rooting using both the growing tip and the single node of the plant.

Surface sterilization of explants: The explants (apical meristem and the single node) were taken from the growing branches of the plants in the western Anbar region of Iraq. They were transported to the laboratory and cleansed with soap and water and rinsed for 30 min under running water. They were then transferred to the isolation room and complete surface sterilization was performed with the excised explant of the apical meristem and the single node immersed separately in a 3% sodium hypo chlorate solution. They were stirred continuously for 5 min, and then washed thrice with sterile, deionized water for 5 min each and rinsed to eliminate any residual effects of the treatment (5).

Preparing the nutritional medium: The medium (MS Murashige and Skoog) (16) from Caisson Company was used at 4.43 gm L^{-1}. Sucrose was added in an amount of 30g and growth regulators added after they had been prepared in the nutrient medium,

the volume completed to 800ml of distilled water, and the components were completely dissolved. The pH was adjusted to 5.7 by adding drops of sodium hydroxide solution (NaOH), then the final volume was completed with 1000ml distilled water and 7gm L^{-1} of agar added. The agar was dissolved with the nutrient medium components by heating the medium's components on a hot plate magnetic stirrer. Following its complete dissolution, it was distributed in special tightly-sealed culture bottles and transferred. The samples were subsequently autoclaved at 121°C at a pressure of 1.04 kg/cm² for 20 min.

Initiation stage: During this stage, the MS nutrient medium was used with combinations of benzyl adenine and kinetin at concentrations of 0.0, 0.5, 1.0, and 1.5 mg L^{-1}. Explants from the growing tip and the single node were used after sterilizing at a length of 0.5-1 cm and planted separately in the growth medium for each growth regulator at twenty replicates for each treatment. The grown tubes were then placed in the growth incubator at a temperature of 25°C, with 16 h of light and 8 h of darkness, for a period of 4 weeks, after which the characteristics were examined for the percentage of dead parts and living explants that did not respond to growth, the vegetative explants, and the live explants that responded to growth.

Multiplication stage: At this stage, the growth regulators BA and Kin were added in different concentrations of 0, 1, 1.5, and 2 mg L^{-1} to the MS nutrient medium which was sterilized as described earlier. The explants' growing tips and single nodes resulting from the culture initiation stage were cultured, each with different growth regulators and lengths of between 1 and 1.5 cm. Ten replicates were used for each treatment and placed in the growth room at a temperature of 25°C for 16 and 8 hours of light and darkness, respectively. After 4 weeks, data was recorded for studied traits such as number of leaves, branch lengths, and number of branches.

Rooting stage: The Murashige and Skoog (MS) medium formulated with the full complement of salts served as the first experimental factor. Shoots obtained from the multiplication stage were then transferred to a fresh MS medium also containing the full complement of salts, and supplemented with the growth regulator IBA as the second factor, at concentrations of 1.0, 0.5, 0.25, 0.0 mg L^{-1} as a factorial experiment to encourage rooting. Data was taken 4 weeks after culture.

Shoots from the multiplication stage were cultured on half-strength MS medium supplemented with IBA at 0, 0.25, 0.5, or 1.0 mgL^{-1} for root induction and the data recorded 4 weeks after planting. Ten replicates were used for each treatment and examined for rooting percentage, root length and number.

Acclimatization stage: The plantlets were taken after they formed the root system, washed thoroughly with water to remove any remains of the nutrient medium and planted in small pots containing a medium consisting of peat moss and sand in a ratio of 1:1. They were sterilized in a laboratory autoclave for 20 min at 121°C and 1.2 kg cm^2 of atmospheric pressure, then covered with a plastic lid and left for two weeks, with the cover pierced at frequent intervals. The plastic covers were gradually removed and the pots transferred to the greenhouse in the same growing medium of peat moss and sand.

Experiment design and statistical analysis: Laboratory experiments were applied according to a completely randomized design (CRD), with ten replicates for each multiplication and rooting treatment. Except for initiation twenty replicates were used. Arithmetic means were compared according to the least significant difference (LSD) test at the 0.05 probability level (6).

Results and Discussion

Culture Initiation Stage: The results in Table 1 show that after culturing the explants of the growing tips, there was a significant effect from adding benzyl adenine BA on the percentage of dead explants, as the 1.5 mg L^{-1} treatment provide the lowest percentage of dead explants at 5%, which did not differ significantly from the 1 mg L^{-1} and 0.5 mg L^{-1} treatments. The comparison group exhibited the highest percentage of dead parts 50%.

There were also no significant differences in traits for the concentrations used in the living explants that did not respond to growth. As for the percentage of living explants achieving growth responses, the results show a significant superiority at 80% for the 1.5 mg L^{-1} treatment compared to the 65% for the 0.5 mg L^{-1} treatment, not significantly different from the former. In comparison the lowest percentage of explants responding to growth in the control treatment was 30%. Table 1 shows the effect of concentrations of BA on the development of the growing apices of capers on the percentage of dead explants, living ones that did not respond to growth, and living parts that responded to growth.

Table 1: Effect of BA concentrations on the shoot tip culture of caper plant on the percentage of dead, non-responsive living, and responsive living explants.

BA Concentrations Mg L^{-1}	Dead Explants %	Living Explants Not Responding to Growth %	Living Explants Responding to Growth %
0	50.0	20.0	**30.0**
0.5	20.0	15.0	**65.0**
1	10.0	30.0	**60.0**
1.5	5.0	15.0	**80.0**
LSD = 0.05	15.6	N.S	**17.8**

Table 2 shows the effects on the percentage of dead explants for the node. The 1.5 mg L^{-1} treatment excelled, with the lowest percentage of dead explants at 5%, followed by the 1 mg L^{-1} and 0.5 mg L^{-1} treatments, which did not differ significantly from the 1.5 mg L^{-1} treatment, achieving 10% and 15%, respectively. The control treatment had the highest percentage of dead parts at 25%. As for the living parts that did not respond to growth, the 1 mg L^{-1} treatment excelled with the lowest percentage at 15%, followed by the 1.5 mg L^{-1} treatment at 35%, not significantly different from the former. The control treatment provided the highest percentage at 55%

The results of explants responding to growth showed significant superiority at the 1 mg L^{-1} treatment 75%, not significantly different from the 60% achieved at 1.5 mg L^{-1}. Meanwhile the control group recorded the lowest percentage at 20%.

Table 2: Effect of BA concentrations on the single node culture of caper plant on the percentage of dead, non-responsive living, and responsive living explants.

BA Concentrations Mg L^{-1}	Dead Explants %	Living Explants Not Responding to Growth %	Living Explants Responding to Growth %
0	25.0	55.0	**20.0**
0.5	15.0	45.0	**40.0**
1	10.0	15.0	**75.0**
1.5	5.0	35.0	**60.0**
LSD = 0.05	11.6	21.2	**20.2**

Table 3 shows the significant effect of kinetin on the shoot tip culture of the caper plant. Parts cultured in the MS nutrient medium had the lowest percentage of dead parts in the 1.5 mg L^{-1} treatment at 5%, which was significantly different from the 0.5 and the 1 mg L^{-1} treatments, which had a percentage of 15%, while the control treatment (without adding kinetin) had the highest percentage of dead parts at 25%.

As for the living explants not responding to growth, the results show the effect of adding kinetin on both the characteristics and percentage, with the 1 and 1.5 mg L^{-1} treatments providing the lowest percentage at 15%, and the 0 and 0.5 mg L^{-1} treatments generating the highest at 25%. The addition of kinetin significantly affected the traits of the living explants responding to growth with the 1.5 mg L^{-1} treatment providing the highest percentage at 80%. This did not differ significantly from the 1 mg L^{-1} treatment (70%) but occurred for the 0.5 mg L^{-1} treatment. The control and 0.5 mg L^{-1} treatment provided percentages of 50% and 60%, respectively.

Table 3: Effect of kinetin concentrations on the single node culture of caper plant on the percentage of dead, non-responsive living, and responsive living explants.

Kinetin Concentrations Mg L^{-1}	Dead Explants %	Living Explants Not Responding to Growth %	Living Explants Responding to Growth %
0	25.0	25.0	**50.0**
0.5	15.0	25.0	**60.0**
1	15.0	15.0	**70.0**
1.5	5.0	15.0	**80.0**
LSD = 0.05	7.9	10.5	**18.5**

As Table 4 shows, the addition of different concentrations of kinetin had an effect on the percentage of dead explants for the node as the 1 mg L^{-1} treatment was significantly superior with the lowest percentage (10%), followed by the 1.5 mg L^{-1} and 0.5 mg L^{-1} treatments at 15% and 20%, respectively. The control treatment recorded the highest percentage of dead explants (25%). In terms of the character and percentage of living explants not responding to growth with the addition of kinetin, the 0.5 and 1 mg L^{-1} treatments provided the lowest percentage at 25% while the 0 and 1.5 mg L^{-1} ones had the highest at 30%. The addition of kinetin had a significant effect on the characteristics of the living explants responding to growth. The treatment with 1 mg L^{-1} kinetin provided the highest percentage at 65%, while that

for 0.5 mg L^{-1} and 1.5 mg L^{-1} kinetin registered 55%. The control treatment had the lowest percentage at 45%.

Table 4: Effect of kinetin concentrations on the single node culture of caper plant on the percentage of dead, non-responsive living, and responsive living explants.

Kinetin Concentrations Mg L^{-1}	Dead Explants %	Living Explants Not Responding to Growth %	Living Explants Responding to Growth %
0	25.0	30.0	**45.0**
0.5	20.0	25.0	**55.0**
1	10.0	25.0	**65.0**
1.5	15.0	30.0	**55.0**
LSD = 0.05	10.6	15.6	**22.6**

The excised explants of the caper (apical meristem and single node) showed varied responses according to the concentrations of BA and kinetin added to the MS medium. The use of BA in initiation, without others, is attributed to its high effectiveness dependent on the number of double bonds in the adenosine loop side chain, where it possesses three double bonds; thus its efficiency is higher in stimulating cell division (4). The reason for the increase in the proportion of responsive living parts in the apical meristem may be attributed to a medium containing 1.5 mg L^{-1} of BA and kinetin to the availability of the ideal proportion of cytokines and to the internal reservoirs of growth regulators such as auxins in the apical meristem of the caper plant. The presence of these regulators causes cell division and increased growth, and cytokinin plays a significant role in stimulating cell division and growth rate acceleration, which increases growth rate.

These results are consistent with (10). Also, the lack of growth response from explants despite their continued viability may be due to differences in hormonal content and cell maturity (15). The reason for their growth response is that cytokinins play a role in breaking apical dominance and stimulating lateral bud growth because it has an active role in RNA and protein synthesis and enzymes in plant cells, and these results are consistent with (3).

The multiplication stage of the apical meristem and single node: The data presented in Table 5 on the multiplication of the apical meristem and the single node shows the significant superiority of the 1.5 mg L^{-1} BA treatment over the others, with the highest number of leaves at 7.30 leaves $plantlet^{-1}$, while that with no growth regulators having the lowest number of leaves (3.50 leaves $plantlet^{-1}$). Also, the treatment with 1.5 mg L^{-1} BA concentration significantly surpassed all others in branch length with an average of 3.78 cm, with the control having the shortest at 1.95 cm.

The results also indicate that the treatment with 1.5 mg L^{-1} BA concentration significantly surpassed all other treatments with the highest average number of branches at 3.50 per plant. At the same time there were no significant differences among the three treatments, with 0 mg L^{-1} recording the lowest number of branches (1.70 branches $plantlet^{-1}$).

Table 5: Effect of concentrations of BA on the multiplication of the apical meristem of the caper plant on the number of leaves, branch length, and number of branches.

BA Concentrations Mg L^{-1}	Number of Leaves (Leaf $Plantlet^{-1}$)	Branch Length Cm	Number of Branches (Branch $Plantlet^{-1}$)
0	3.50	1.95	**1.70**
1	4.30	2.00	**2.20**
1.5	7.30	3.78	**3.50**
2	4.70	2.65	**2.30**
LSD = 0.05	1.63	0.58	**0.77**

Table 6 presents data on the impact of various treatments on the nodal multiplication of caper plants. The highest number of leaves (5.60 leaves $platlet^{-1}$) occurred when treated with 1.5 mg L^{-1}, which is not significantly different from the 2 mg L^{-1} (5.30 leaves $platlet^{-1}$) concentration, while the comparison treatment recorded the lowest at 1.70 leaves $platlet^{-1}$. The analysis shows significant superiority in branch length per node (3.46 cm) at a concentration of 1.5 mg L^{-1} while the control treatment achieved the lowest average at 1.64 cm. In regard to average number of branches, the 1.5 mg L^{-1} treatment was the most significant at 4.90 branches $platlet^{-1}$, while the control treatment had the lowest at 1.50 branches $platlet^{-1}$.

Table 6: Effect of concentrations of BA on the multiplication of the single node of the caper plant on the number of leaves, branch length, and number of branches.

BA Concentrations Mg L^{-1}	Number of Leaves (Leaf $Plantlet^{-1}$)	Branch Length Cm	Number of Branches (Branch $Plantlet^{-1}$)
0	1.70	1.64	**1.50**
1	4.30	2.19	**1.90**
1.5	5.60	3.46	**4.90**
2	5.30	2.22	**2.80**
LSD = 0.05	1.28	0.63	**0.64**

Table 7 shows the effects of kinetin on the apical meristem, with the 2 mg L^{-1} concentration significantly outperforming other treatments at 5.90 leaf plantlet-1 and the lowest seen under the control treatment at 1.70 leaf $plantlet^{-1}$. The results also indicate a major difference in branch lengths with the 3.22 cm average for the 2 mg L^{-1} concentration treatment significantly outperforming the control at 0.70 cm1. Also, the 2 mg L^{-1} treatment was significantly superior to the others at 2.00 branch $plantlet^{-1}$, with the control recording the lowest at 1.10.

Table 7: Effect of concentrations of kinetin on the multiplication of the apical meristem of the caper plant on the number of leaves, branch length, and number of branches.

Kinetin Concentrations Mg L^{-1}	Number of Leaves (Leaf $Plantlet^{-1}$)	Branch Length Cm	Number of Branches (Branch $Plantlet^{-1}$)
0	1.70	0.70	**1.10**
1	2.90	2.06	**1.40**
1.5	2.20	2.37	**1.80**
2	5.90	3.22	**2.00**
LSD = 0.05	1.38	0.57	**0.69**

Table 8 illustrates the kinetin effect on the multiplicated nodes. The 2 mg L^{-1} application achieved the highest multiplication with the largest number of leaves at 6.80 leaves $plantlet^{-1}$ while the control had the lowest (1.50 leaves $plantlet^{-1}$). Furthermore, it demonstrates significant differences in branch lengths with the 2 mg L^{-1} treatment substantially outperforming other treatments at 3.95 cm. The lowest average branch length was for the control application at 1.43 cm. Additionally, media fortified by 2 mg L^{-1} caused an increase in branches of 2.20 branch $plantlet^{-1}$ while the control had the least at 1.10.

Table 8: Effect of concentrations of kinetin on the multiplication of the single node of the caper plant on the number of leaves, branch length, and number of branches.

Kinetin Concentrations Mg L^{-1}	Number of Leaves (Leaf $Plantlet^{-1}$)	Branch Length Cm	Number of Branches (Branch $Plantlet^{-1}$)
0	1.50	1.43	**1.10**
1	2.00	1,94	**1.40**
1.5	3.30	2.76	**1.60**
2	6.80	3.95	**2.20**
LSD = 0.05	1.38	0.58	**0.66**

Cytokines are added during the vegetative multiplication stage of many plant species due to their effectiveness in stimulating axillary buds without cutting the growing apex, as they resist the action of auxin (21). This is in addition to their importance in stimulating cell division and adventitious branches, as mentioned above. This explains why adding BA and kinetin in concentrations of 1.5 and 2 mg L^{-1} produced the optimal number of leaves for both the apical meristem and the single node, results that are in line with the findings of Musallam et al. (17).

The increase in the number of branches, especially for the concentration of 1.5 mg L^{-1} of BA in a single node, may be attributed to the positive effect of BA in increasing the number of branches and leaves and reducing apical dominance, as well as its role in attracting and accumulating metabolic substances at lateral shoot sites to initiate and stimulate their growth, directly aiding in RNA, protein, and chlorophyll synthesis (13). Adding BA also leads to a hormonal imbalance in the meristematic areas rich in auxins, disrupting apical dominance and the subsequent stimulation of lateral bud growth (4).

Rooting stage: Table 9 shows the significant effect of the auxin IBA on the rooting of the branches resulting from the growing apex and node of the caper plant cultured in vitro in the MS medium with full salt concentration. Most treatments provide rooting for these branches, but at different percentages. Treating with 0.5 mg L^{-1} IBA provides the highest rooting rate (70%), followed by 1 mg L^{-1} (60%) compared to the lowest at 30% for the control. However, no significant differences were found in either the number of roots or their lengths.

Table 9: Effect of IBA concentrations on rooting of caper plant branches on MS medium with full salt concentration (rooting percentage, number of roots, root length).

IBA Concentrations Mg L^{-1}	Rooting Rate %	Number of Roots (root $plantlet^{-1}$)	Root Length Cm
0	30.0	1.10	**1.01**
0.25	50.0	1.00	**1.12**
0.50	70.0	2.20	**1.61**
1.0	60.0	2.50	**1.72**
LSD = 0.05	18.9	N.S	**N.S**

In regard to the effect of auxin IBA on rooting of branches in an MS medium at half the concentration of salts, Table 10 show that most treatments resulted in rooting of the branches represented by the apical meristem and node of the caper plant in vitro. The treatment with a 0.5 mg L^{-1} IBA concentration outperformed all others, exhibiting a rooting rate of 80%. It did not differ statistically from the 0.25 and 1.0 mg L^{-1} IBA treatments, which both recorded 70% rooting rates.

The control treatment provided the lowest rooting rate at 50%. There was a significant effect on root numbers in adding IBA, with the 1 mg L^{-1} treatment producing the highest average number of roots at 3.70 roots plantlet-1 followed by the lowest for the control at 1.10. The highest average root length at 2.91 cm was generated by the 1 mg L^{-1} followed by 0.5 mg L^{-1} at 2.64 cm, which is not significantly different from the former. The 0.25 mg L^{-1} treatment provided the lowest average root length at 1.03 cm.

Table 10: Effect of IBA concentrations on rooting of caper plant branches on MS medium with half salt concentration (rooting percentage, number and length of roots).

IBA Concentrations Mg L^{-1}	Rooting Rate %	Number of Roots (Root $Plantlet^{-1}$)	Root Length Cm
0	50.0	1.10	**1.51**
0.25	70.0	1.60	**1.03**
0.50	80.0	2.00	**2.64**
1.0	70.0	3.70	**2.91**
LSD = 0.05	14.8	1.56	**1.35**

As seen in Tables 9 and 10, an MS medium at half the standard salt concentration resulted in a greater rooting percentage, a higher number of roots per plant, and longer roots than full-strength medium. The reduced levels of salts in the nutrient medium helps stimulate growth and differentiation; reducing the salt concentration by half or a quarter may lead to stimulating the formation of cultured branches as this enhances the strength of the effect of carbohydrates (sucrose) thus stimulating root growth (2). Increases in root lengths also occurred from the reduction in salt concentrations by half, and this is attributed to the phenomenon of nutrient tropism, which leads to competition for nutrients, thus stimulating the roots to grow in the medium for nutrient intake (7). Auxin is also important for the formation of roots on branches, as the division of root initials depends on its presence in transforming specialized cells into meristematic cells. This enables the formation of the adventitious root meristem, which divides to form roots.

These results agree with those of Elmaghrabi et al. (10). Using 1 mg L^{-1} provides the best response in the number of roots and root length of the caper plant, as high concentrations of auxin may reduce the rate of root formation due to the formation of ethylene in plant tissues, which leads to the inhibition of its formation in plant tissues (11).

Acclimatization of the resulting plantlets: The acclimatization procedures were successful for the plants formed from the treatments that achieved the best results in micropropagation, especially those that obtained the best rooting rates, using a mixture of peat moss and sand (1:1) and incubated in the growth room for two weeks. The survival rate of the remaining plantlets was 80%, after which they were transferred to the greenhouse.

Conclusions

As a result of this study, a new protocol was established for in vitro micropropagation of plants (apical and single-node meristems) of C. spinosa, based on concentrations of 1.5 mg L^{-1} of BA for the initiation and multiplication stages and 0.5 mg L^{-1} of IBA for the rooting stage for explants excised from wild plants. This protocol can be used for its propagation in Iraq due to its importance in bearing stress and producing medical materials.

Supplementary Materials:

No Supplementary Materials.

Author Contributions:

Author T. F. Farhan; methodology, writing—original draft preparation, Author Y. S. Sekhi writing—review and editing. All authors have read and agreed to the published version of the manuscript.

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