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An Efficient Protocol for Successful in Vitro Propagation of *Cardiospermum halicacabum*: An Ornamental Plant with Medicinal Powers Growing in the Jordanian Environment

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ABSTRACT

Cardiospermum halicacabum is an ornamental plant known for its significant medicinal benefits. It is utilized in the treatment of various ailments, including rheumatism, neurological disorders, and snakebites. However, overgrazing and poor seed germination are the main constraints for *C. halicacabum* propagation and sustainability. Tissue culture can be applied as an efficient technique for increasing and conserving this plant. In this research, the effect of several growth regulators and pH levels on *C. halicacabum* micropropagation was investigated. For shoot proliferation growth regulators (Benzyl Amino Purine (BAP), zeatin, and kinetin) at different levels (0.0, 0.4, 0.8, 1.2, 1.6, and 2.0 mg/L) were tested to enhance shoot development in *C. halicacabum*. Benzyl Amino Purine (BAP) was found to be the most effective hormone for shoot multiplication at (0.8 mg/L) BAP, which produced 3.89 shoots. Meanwhile, pH ranging from 4.0 to 6.0 did not show any significant difference related *to in vitro* propagation. For rooting, indole acetic acid (IAA), naphthalene acetic acid (NAA), and indole-3-butyric acid (IBA) at (0.0, 0.4, 0.8, 1.2, 1.6, and 2.0 mg/L) were tested. IAA was the best growth regulator used for rooting of *C. halicacabum.* Most plantlets (90%) were acclimatized under greenhouse conditions. Our data demonstrate a successful in vitro propagation method of *C. halicacabum*. This would encourage further research work on *C. halicacabum*, such as investigating its active ingredients and antimicrobial powers.

Keywords: *C. halicacabum*, In vitro propagation, Jordan, Medicinal plant, Rooting, Tissue culture.

INTRODUCTION

Medicinal plants are rich beneficial aids that play an important role in well-being schemes for both humans and animals (Ferrara, 2018; Osman et al., 2021; Shatnawi et al., 2022; Asigbaase et al., 2023). Therefore, it is desired for remedial plants to evaluate their photochemistry to determine the possibility that these original products can be used for the medication (Menichini et al., 2014). The development of industrial technology and the rise in occupants have put great pressure on natural resources, and so medicinal plants are declining or disappearing and becoming endangered (Saifan et al., 2024). Medicinal plants moving from marginal to the majority are used by people who are looking for medications and well-being (Ferrara, 2018).

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Cardiospermum halicacabum L. belongs to the *Sapindaceae* family, and is recognized as a "balloon vine". This plant is an energetic, remedial climber and spreads in hot climatic regions (America, Africa, and Asia) (Wei et al., 2011). *C. halicacabum* is a herbaceous climber plant that is used as food and eaten as a vegetable (leaves and young shoots). (Rao et al., 2006; Urdampilleta et al., 2013; Elangovan et al., 2022). *C. halicacabum* plant parts, such as leaves, roots, and seeds, have previously been employed for medication because of their curative property (Shekhawat et al., 2012; Urdamilleta et al., 2013; Gaziano et al., 2019; Suresh et al., 2023). *C. halicacabum* is used for the treatment of rheumatism, nervous diseases, stiffness of the limbs, and snakebites (Raza et al., 2013; Vijayakumar & Kumar, 2021). In addition, the *C. halicacabum* plant has been employed as an "anti-inflammatory, antipyretic, analgesic, antiparasitic, as well as an effective nontoxic antifertility herb" (Boonmars et al., 2005; Shekhawat et al., 2012). Other studies reported that *C. halicacabum* has significant analgesic, anti-inflammatory, antifilarial, antibacterial, antidiarrhoeal, antipyretic, antiparasitic, antimalarial, and antioxidant activities (Rao et al., 2006; Meesil et al., 2025).

Conventional propagation of *C. halicacabum* by seed is usually slow (Ahmad & Anis, 2007a; Shekhawat et al., 2012). Clonal propagation is the most proper technique that is used to propagate plants enormously (Perez-Tornero et al., 2000; Nas & Read, 2004; Al-Ajlouni et al., 2015; Al-Qudah et al., 2023). Tissue culture is a propagation system that permits the development of massive plant material under *in vitro* conditions in a short time (Shatnawi et al., 2021). The tissue culture propagation method permits the propagation of plant material with great proliferation rates (Shibli et al., 2006; Faisal et al., 2010; Shatnawi et al., 2011, 2019). Tissue culture can also play a significant role in *C. halicacabum* biotechnology (Ahmad & Anis, 2007; Shekhawat et al., 2012).

Meanwhile, the development of an in vitro propagation protocol for *C. halicacabum* is of unlimited significance for the improvement of this important medicinal plant. Few reports are available on the *in vitro* propagation of this important plant (Ahmad & Anis, 2007 b; Shekhawat et al., 2012; Kumar et al., 2016; Jahan & Anis, 2009 & 2018; Thaniarasu & Logeshwari, 2021). For example, a successful technique for the rapid micropropagation of *C. helicacabum* has been established, as reported by Thomas and Maseena (2006) which involved the regeneration of plants from calli from explants in Murashige and Skoog (MS) medium plus 2,4-dichlorophenoxy acetic acid (2,4-D). Also, Jahan & Anis (2009) had developed an effective protocol for the micropropagation of *Cardiospermum halicacabum* through axillary buds using a medium enriched with 0.3 µM TDZ. In Jordan, our research represents the first attempt at micropropagation of *C. halicacabum*, which grows in the gardens of Jordanians as an ornamental plant to improve propagation chances for this valuable plant.

Materials and Methods

Establishment of mother stock

"Seeds from mature fruits of *Cardiospermum halicacabum* were collected in September from Al Husun, Irbid-Jordan (Latitude and longitude are: **32.551445 N, 35.851479 E).** Seeds were sterilized according to Shatnawi et al. (2012) methods. Seeds of *C. halicacabum* were washed thoroughly with running tap water for 15 min. Then the seeds were rinsed with sterile distilled water and treated with 4% (w/v) sodium hypochlorite for 20 min. This step was followed by rinsing 4 times with autoclaved, sterilized distilled water to remove any traces of sterilant. Seeds were then incubated on respective germination medium (8 g/L agar and water). Only 58% of seeds were able to germinate. After the seed germinated, the resulting microshoots were subcultured onto Murashige & Skoog's (1962) (MS) medium.

Microcultures were incubated in the growth chambers at 24 ± 2 ºC with a 16 h photoperiod and photosynthetic photon flux density (PPFD) of 50 µmol $m^{-2}s^{-1}$ supplied by cool white fluorescent lamps. After six weeks, microshoots were cultivated onto flasks where each of which contained 80 ml MS medium (Murashige & Skoog's, 1962) plus 30 g/L sucrose and 0.01 mg/L benzyl amino purine (BAP), and maintained under the same

conditions to generate enough plant material before experimentation."

Shoot proliferation

Effect of benzyl amino purine (BAP), zeatin, and kinetin

Microshoots (10 mm) were subcultured into MS medium containing either BAP, zeatin or kinetin at various concentrations (0.0, 0.4, 0.8, 1.2, 1.6, and 2.0 mg/L). Each treatment consisted of 5 flasks (replicates) with 4 microshoots/flask, and each experiment was repeated twice. Data were collected after six weeks for the number of shoots, shoot length, shoot fresh weight, and dry weight of the shoot.

Effect of pH

Microshoots, 10 mm in length, were transferred onto MS proliferation medium containing 0.2 mg/L BAP and 2.0 mg/L GA_3, with various pH (4.0, 4.5, 5.0, 5.5, 5.8, and 6.0) values based on the best results that were obtained from the above experiment. Data were collected after six weeks.

***In vitro* root formation**

Microshoots (10 mm) were cultivated on MS medium consisting of either indole-3-butyric acid (IBA), indole acetic acid (IAA), or naphthalene acetic acid (NAA) at different concentrations (0.0, 0.4, 0.8, 1.2, 1.6, and 2.0 mg/L). Six weeks later, data were collected for the number of shoots, shoot length, root number, root length, and root percentage.

***Ex vitro* acclimatization**

The in vitro grown plantlets were moved to a growth room, then rooted microshoots were taken out from the medium, soaked in a water bath at room temperature (24 ± 2 °C), and anchored on plastic pots (6 x 6 x 6 cm) containing a sterile mixture of 1 perlite: 1 peat and covered with a transparent plastic bag (15 x 20 cm). By making a hole (1-2 mm) in the bag, the humidity was reduced gradually. The size of the hole was increased every 1-2 days for over 14- 20 days. After six weeks percentage of survival of acclimatized plants was recorded.

Statistical analysis

In this study, the experiments were prepared as a completely randomized design (CRD). Each treatment consisted of 5 flasks with 4 microshoots/flask, and the results were exposed to the ANOVA test. Tukey HSD test at p= 0.05 was used for mean separation. Data were analyzed using SPSS (SPSS, 2007).

Results

Effect of different types and levels of plant growth regulators on the growth of *C. halicacabum* under *in vitro* conditions

Effect of benzyl amino purine (BAP)

In vitro microshoot proliferation of *C. halicacabum* was affected by BAP concentration (Table 1). Results obtained from the control treatment (hormone-free MS medium) showed that 1.40 microshoots were developed after six weeks' inoculation, while the length of the shoot was 1.8 cm (Table 1). On the other hand, adding 0.8 mg/L BAP to the MS media has resulted in a significant increase in microshoot proliferation (3.89 microshoots/ explant). Meanwhile, explants grown in MS medium containing higher BAP levels didn't improve the development of new shoots (Table 1). Furthermore, a significant increase was found in fresh weight in response to BAP levels of (0.8, 1.2 mg/L), while no significant differences were found in dry weight in response to any BAP treatments compared to the control (Table 1).

Table 1: Effects of various benzyl amino purine (BAP) concentrations on *in vitro* growth of *Cardiospermum halicacabum.*

BAP (mg/L)	Number of new shoots/explant	Shoot length (cm)	Fresh weight (g)	Dry weight (g)
0.0	1.410 ± 0.010 c	1.800 ± 0.078 d	0.080 ± 0.005 b	0.018 ± 0.000 a
0.4	1.410 ± 0.012 c	2.440 ± 0.036 c	0.080 ± 0.004 b	0.019 ± 0.000 a
0.8	3.890 ± 0.037a	2.900 ± 0.038 a	0.130 ± 0.013 a	0.019 ± 0.000 a
1.2	1.570 ± 0.011 b	2.800 ± 0.049 b	0.120 ± 0.011 a	0.018 ± 0.000 a

1.6	1.580 ± 0.013 b	2.480 ± 0.031 c	0.080± 0.003 b	0.018 ± 0.000 a
2.0	1.580 ± 0.021 b	1.830 ± 0.080 d	0.080 ± 0.005 b	0.019 ± 0.000 a

Values represent means± standard deviation (SD). Means within each column were analyzed separately. Means with different letters are significantly different according to Tukey's HSD test at a probability level of 0.05.

Effect of kinetin

Data showed that microshoot development was significantly affected by kinetin concentration (Table 2). The maximum number of new shoots formed (3.7) was gained on MS media with 0.8 mg/L kinetin (Table 2). A Similar trend was also found in shoot length, as kinetin treatment higher than (0.8 mg/L) didn't support shoot elongation. (Table 2). However, kinetin at 0.8 mg/L was found to be the best for microshoot proliferation and elongation in *C. halicacabum* compared to all other treatments. Meanwhile, ANOVA analysis revealed that dry weight was not affected by adding kinetin to the media (Table 2).

Table 2: Effects of various kinetin concentrations on *in vitro* growth of *Cardiospermum halicacabum.*

Kinetin (mg/L)	Number of new shoots/explant	Shoot length (cm)	Fresh weight (g)	Dry weight (g)
0.0	1.850 ± 0.043 d	1.850 ± 0.134 b	0.080 ± 0.004 b	0.018 ± 0.000 a
0.4	2.500 ± 0.040 c	2.400 ± 0.334 a	0.100 ± 0.010 a	0.018 ± 0.004 a
0.8	3.700 ± 0.051 a	2.350 ± 0.313 a	0.103± 0.012 a	0.019 ± 0.000 a
1.2	2.850 ± 0.046 b	1.850 ± 0.134 b	0.100± 0.010 a	0.019 ± 0.002 a
1.6	1.850 ± 0.051 d	1.800± 0.132 b	0.100 ± 0.010a	0.018 ± 0.000 a
2.0	1.850 ± 0.045 d	1.800 ± 0.133 b	0.100 ± 0.011a	0.019 ± 0.001 a

Values represent means± standard deviation (SD). Means within each column were analyzed separately. Means with different letters are significantly different according to Tukey's HSD test at a probability level of 0.05.

Effect of zeatin

The presence of zeatin in the culture medium increased the rate of shoot proliferation. The maximum number of new shoots (3.15 microshoots) was recorded at the level of 1.2 mg/L zeatin, after a six-week growth period, while adding higher zeatin levels didn't support the development of new shoots (Table 3). Shoot length was influenced by adding zeatin at different concentrations to the media. Maximum shoot length (2.30 cm) was observed after six-week-old cultures on MS medium supplemented with 1.6 mg/L zeatin (Table 3; Fig. 1). Data revealed that fresh and dry weights were not significantly affected by zeatin treatments (Table 3).

Table 3: Effects of different zeatin levels on *in vitro* growth of *Cardiospermum halicacabum.*

Zeatin (mg/L)	Number of new shoots/explant	Shoot length (cm)	Fresh weight (g)	Dry weight (g)
0.0	1.400 ± 0.040 d	1.850 ± 0.052 c	0.080 ± 0.001 a	0.018 ± 0.001 a
0.4	1.900 ± 0.023 c	1.900 ± 0.044 c	0.080 ± 0.003 a	0.018 ± 0.000 a
0.8	2.950 ± 0.047 b	1.900 ± 0.040 c	0.090 ± 0.001 a	0.019 ± 0.001 a
1.2	3.150 ± 0.105 a	2.150 ± 0.023 b	0.090 ± 0.003 a	0.019 ± 0.000 a
1.6	1.900 ± 0.020 c	2.300 ± 0.030 a	0.090 ± 0.001 a	0.020 ± 0.003 a
2.0	1.900 ± 0.022 c	2.250 ± 0.032 a	0.090 ± 0.005 a	0.019 ± 0.004 a

Values represent means± standard deviation (SD). Means within each column were analyzed separately. Means with different letters are significantly different according to Tukey's HSD test at a probability level of 0.05.

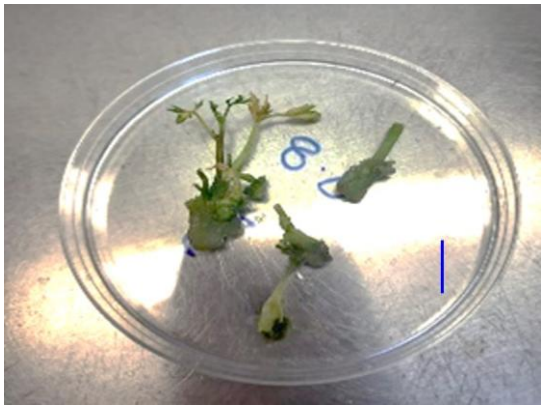

Figure 1: shoot multiplication in *Cardiospermum halicacabum*, which was grown on MS plus 1.2 mg/L zeatin. The scale bar represents 1.0 cm.

Effect of pH on growth of *C. halicacabum* under *in vitro* conditions

Based on the recorded results, the value of medium pH had affected all measured growth parameters (Table 4). Moreover, most growth parameters including the number of new shoots (3.90), in addition to fresh and dry weights, were maximized at a pH level of 6.0 (Table 4). Meanwhile, no signs of hyperhydricity were noticed on any plant that was grown at pH levels of 5.0 or more (Table 4).

Table 4: Effects of different pH levels on *in vitro* growth of *Cardiospermum halicacabum.*

pH level	Number of new shoots/explant	Shoot length (cm)	Fresh weight (g)	Dry weight (g)	Hyper-hydricity
4.0	2.800±0.027c	2.230±0.022 c	0.085± 0.001 c	0.019± 0.001 b	+
4.5	2.800±0.024c	2.690 ±0.017 b	0.095± 0.004 b	0.019± 0.003 b	+
5.0	3.500±0.030b	2.690±0.020 b	0.095±0.001 b	0.019 ± 0.000b	-
5.5	3.500±0.028b	2.900±0.027 a	0.096 ± 0.003 b	0.019 ± 0.001b	-
5.8	3.800±0.042a	2.900 ±0.025 a	0.097 ± 0.001 b	0.019 ± 0.002b	-
6.0	3.900±0.047a	2.690±0.010 b	0.102± 0.004 a	0.020± 0.002 a	-

Values represent means± standard deviation (SD). Means within each column were analyzed separately. Means with different letters are significantly different according to Tukey's HSD test at a probability level of 0.05. (+): presence of hyperhydricity, (-): absence of hyperhydricity.

Effect of different types and levels of growth regulators on rooting of *Cardiospermum halicacabum* under *in vitro* conditions

Effect of indole-3-butyric acid (IBA)

Using IBA at 0, 1.6, and 2.0 mg/L, a maximum number of shoots were developed (1.25), with a short length of 3.5 cm. The maximum number of roots (3.45) was formed in media with 0.8 or 1.2 mg/L IBA, with a root length of 2.5 cm (Table 5). Roots were developed on microshoots after six weeks on MS medium having IBA. A callus was formed at the bottom end of the microshoot on an MS medium with or without IBA.

Table 5: Effect of different indole-3-butyric acid (IBA) concentrations on root formation of *in vitro* growth of *Cardiospermum halicacabum.*

IBA (mg/L)	Shoot length (mm)	Number of roots/explant	Root length (cm)	Number of new shoots/explant	Rooting %	Callusing
0.0	2.30 ± 0.20c	1.40± 0.29d	1.50±0.26c	1.25±0.03a	40±6.70 b	+
0.4	3.50 ± 0.29a	3.10± 0.26 b	1.50±0.21c	1.00±0.09c	100±0.00a	+
0.8	3.50± 0.32a	3.45± 0.31 a	2.50 ±0.33a	1.13±0.05b	100±0.00a	+

1.2	3.37±0.20 ab	3.45 ± 0.27 a	2.50 ±0.29a	1.13±0.02b	100±0.00a	+
1.6	2.30 ± 0.24c	3.10± 0.26 b	2.60 ±0.31a	1.25±0.07a	100±0.00a	+
2.0	3.37±0.27 ab	2.90± 0.22 c	2.30±0.27a	1.25±0.03a	100±0.00a	++

Values represent means± standard deviation (SD). Means within each column were analyzed separately. Means with different letters are significantly different according to Tukey's HSD test at a probability level of 0.05. (+) represent < 5 mm callus diameter, (++) represent 5-10 mm callus diameter."

Effect of IAA

The highest length of microshoots (3.5 cm) was detected on MS medium having 0.8 mg/L IAA. While highest number of roots (7.90) was attained on MS medium augmented with 1.2 mg/L IAA (Table 6). With the supplement of IAA root formation percentage increased. Moreover, maximum root formation (100%) was identified on MS medium having 0.4 and 1.2 mg/L of IAA (Table 6). In addition, rooting percentage was recorded on microshoots after six weeks on MS medium with IAA, where callus formed at the bottom end of the microshoot.

Table 6: Effects of different concentrations of indole acetic acid (IAA) on root formation of *in vitro* grown *Cardiospermum halicacabum.*

IAA (mg/L)	Shoot length (cm)	Number of roots/explant	Root length (cm)	Number of new shoots/explant	Rooting %	Callusing
0.0	2.30 ± 0.24d	1.40± 0.21 d	1.50 ±0.26d	1.20±0.07 d	40±9.80 d	+
0.4	3.10 ± 0.21b	3.30± 0.37 b	2.80 ±0.35c	1.31 ±0.04c	100±0.00a	++
0.8	3.50±0.28a	3.30 ± 0.31b	3.80 ±0.26b	1.94±0.15 a	90±6.30 b	++
1.2	3.1± 0.23 b	7.90± 0.97 a	5.70 ±0.43a	1.81 ±0.11b	100±0.00a	+++
1.6	2.80± 0.20 c	3.30 ± 0.28b	2.80 ±0.27c	1.20 ±0.10 d	80±5.47c	+
2.0	3.50 ± 0.31a	2.90 ± 0.23c	2.80 ±0.30c	1.31±0.06 c	80±6.10c	+++

Values represent means± standard deviation (SD). Means within each column were analyzed separately. Means with different letters are significantly different according to Tukey's HSD test at a probability level of 0.05. (+) represent < 5 mm callus diameter, (++) represent < 10 mm callus diameter and (+++) represent > 10 mm callus diameter.

Effect of NAA

Using NAA at 2.0 mg/L, the number of induced roots was 3.5 with a root length of 2.7 cm. The highest length of microshoots (1.65 cm) was detected on the MS medium having 2.0 mg/L NAA. The highest number of roots (6.90) was attained on MS medium augmented with 1.2 mg/L NAA (Table 7). Using NAA at a concentration of 0.4 to 1.6 mg/L resulted in a 100% root formation percentage (Table 7; Fig. 2). The highest length of microroots (5.9 cm) was detected on the MS medium having 1.2 mg/L NAA. In addition, root induction was observed on microshoots after two weeks on an MS medium having NAA (Figure 2). A callus was formed at the bottom end of the microshoot on the MS medium containing NAA.

Table 7: Effects of different naphthalene acetic acid (NAA) concentrations on root formation of *in vitro* grown *Cardiospermum halicacabum.*

NAA (mg/L)	Shoot length (cm)	Number of roots/explant	Root length (cm)	Number of new shoots/explant	Rooting %	Callusing
0.0	2.20 ± 0.26a	1.40± 0.31 c	1.50± 0.26 d	1.25± 0.21b	40± 6.70c	+
0.4	1.56 ± 0.27b	3.90 ± 0.24b	2.80 ± 0.22c	1.25± 0.25 b	100±0.00a	++

0.8	1.56± 0.24 b	3.90 ± 0.27b	4.30 ± 0.34b	1.90 ± 0.29a	100±0.00a	+++
1.2	1.30 ± 0.20c	6.90 ± 0.52a	5.90± 0.47 a	1.00 ± 0.05c	100±0.00a	+++
1.6	1.30± 0.22 c	6.90 ± 0.47a	2.80 ± 0.24c	1.00 ± 0.00c	100±0.00a	+++
2.0	1.65 ± 0.24b	3.50 ± 0.26b	2.80 ± 0.27c	1.07 ± 0.06c	90± 6.30b	+++

Values represent means± standard deviation (SD). Means within each column were analyzed separately. Means with different letters are significantly different according to Tukey's HSD test at a probability level of 0.05. "(+) represent < 5 mm callus diameter, (++) represent < 10 mm callus diameter and (+++) represent > 10 mm callus diameter.

Figure 2: Root development in *C. halicacabum*, which was grown on MS supplied with 1.2 mg/L NAA. The scale bar represents 1.0 cm.

Hardening

Rooted plantlets were moved to another growth room (room temperature 25±1°C under light) where plastic pots filled with a mixture of 1:2 (Peat: Perlite). Two to three weeks later, 90 % of rooted plantlets survived the acclimatization procedure. Data was taken after six weeks for the percentage of plantlets that were able to survive and remain healthy after the hardening process. Then, the acclimatized plantlets were transferred to the greenhouse for further growth and development (Figure 3).

Figure 3: Hardening stages of *Cardiospermum halicacabum*. (A): After 2 weeks. (B) After 4 weeks. (C) After 6 weeks.

Discussion

Effect of different types and levels of plant growth regulators on the shooting of *C. halicacabum* under *in vitro* conditions

The obtained data showed that increasing the concentration of BAP had increased shoot number and shoot length in *C. halicacabum*, and the (0.8 mg/L) level of BAP had produced the maximum number of shoots (3.89 shoots) (Table 1). In many related studies, BAP was shown to be an ideal hormone for shoot multiplication in many plants such as, *C. halicacabum*, *Moringa peregrina*, and *Paronchia argentea* (Esther & Robinson, 2013; Alrayes et al., 2019; Shatnawi et al., 2021). Similar observations were also reported in *Ruta graveolens* by Al-Ajlouni et al. (2015), and *Capparis spinosa* (Al-Mahmood et al., 2012).

Shoot length varied in response to the different BAP treatments (Table 1). In this study, the maximum shoot length (2.9 cm) was recorded at 1.6 mg/L BAP. Similar trend was reported in previous findings of (Esther & Robinson, 2013; Al Shhab et al., 2021; Shatnawi et al., 2022).

Fresh and dry weights were improved when the microshoots were grown in MS media containing either BAP or kinetin compared to the control. These results agreed with previous findings in *Moringa peregrine* (Alrayes et al., 2019) and *Ruta graveolens* (Al Shhab et al., 2021) treated with similar hormones. In our study, the improvement in both fresh and dry weights could be attributed to the ability of both hormones to induce the development and growth of more new microshoots in addition to increasing shoot length, which might have positively improved values of both fresh and dry weights (Table 1, 2,). However, our data indicated that the types and concentrations of the growth regulators selected in

this study were good choices for successful micropropagation of *C. halicacabum.*

pH and *in vitro* growth

At a pH range from 5.0–5.5, microshoots were growing normally. The best growth parameters were recorded at pH of 5.8 (Table 4). In various research articles, the medium pH has been adjusted to promote growth in certain plant species. For instance, Huang and Liu (2003) found that Tetraploid black exhibited the highest growth rate at a pH range of 5.6 to 6.0, whereas Erqiao black achieved its optimal growth rate at a pH of 6.4. The medium pH was reported to influence the physical strength or hardness of the medium, as medium pH could affect the membrane permeability, respiratory metabolism, polyamine metabolism, protein synthesis, the activity of hormones, and the growth and development of plant material (Wetzstein et al., 1994; Harbbage et al., 1998; Martin et al., 2011; Shatnawi et al., 2022). Our data showed that setting the pH of the growth media to (5.8-6.0) had a positive effect on all growth parameters. Nutrient availability for the plantlets might have been improved due to these pH values (5.8-6.0), which might cause this increase in growth parameters of *C. halicacabum.*

Root formation

Data presented in Tables 5, 6, and 7 showed the response of *C. halicacabum* to *in vitro* rooting using MS media supplemented with different concentrations of IBA, IAA, and NAA. The results revealed that IAA at 1.2 mg/L produced a maximum number of roots. Similar observations were reported in *Moringa peregrina* (Alrayes et al., 2019) and *Paronchia argentea* (Shatnawi et al., 2021). Callus was developed at the bases of *C. halicacabum* microshoots when treated with either *IBA*, IAA, or NAA (Tables 5, 6, 7). These growth regulators were also used by Thaniarasu & Logeshwari (2021), which were all succeeded in the enhancement of callus growth and development in *C. halicacabum.* Our data indicated why adding auxins to the growth media is highly recommended for rooting in tissue-cultured plants, as they play a crucial role in stimulating cell division, enhancing cell elongation, and initiating roots and callus formation in culture environments.

Conclusions

C. halicacabum possesses distinguished medicinal properties. Tissue culture allowed us to develop a reliable protocol for micropropagation of this plant using MS media with 0.8 mg/L BAP for the highest microshoot proliferation rate, media pH of 5.8, in addition to 1.2 mg/L IAA for rooting, followed by acclimatization under normal growth in the greenhouse. The next step is to apply this protocol for the production of huge amounts of this medicinal plant that would permit screening the types and amounts of the active ingredients of *C. halicacabum* and how to enhance their production in vitro.

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بروتوكول فعال لنجاح اكثار نبات ال:Cardiospermum halicacabum- نبات زينة ذو قوى طبية ينمو في البيئة الأردنية

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ملخص

halicacabum هو نبات زينة معروف بفوائده الطبية الهامة. يتم استخدامه في علاج العديد من الأمراض، بما في ذلك الروماتيزم والاضطرابات العصبية ولدغات الثعابين. لكن الرعي الجائر وضعف إنبات البذور تشكل العوائق الرئيسية لتكاثر *C. halicacabum* واستدامته. يمكن تطبيق زراعة الأنسجة كتقنية فعالة لإكثار هذا النبات والحفاظ عليه. في هذا البحث، تم دراسة تأثير العديد من منظمات النمو ومستويات الرقم الهيدروجيني على تكاثر *C. halicacabum*. بالنسبة لتكاثر السويقات، تم اختبار منظمات النمو (بنزيل أمينو بورين (BAP) والزياتين والكاينتين) بمستويات مختلفة (0.0 و0.4 و0.8 و1.2 و1.6 و2.0 مجم / لتر) لتعزيز انتاج السويقات. وقد تبين أن البنزيل أمينو بورين (BAP) هو الهرمون المثالي لتكاثر السويقات عند (0.8 مجم/لتر). وفي الوقت نفسه، اظهر الرقم الهيدروجيني 6.0 اثرا إيجابيا على نمو النبات داخل الأنابيب. بالنسبة للتجذير العرضي، تم اختبار حمض إندول الأسيتيك (IAA) وحمض النفثالين الأسيتيك (NAA) وحمض إندول-3-بيوتريك (IBA) عند (0.0، 0.4، 0.8، 1.2، 1.6، 2.0 مجم/لتر). حيث تبين أن حمض إندول الأسيتيك هو الأفضل لتجذير *C. halicacabum*. كما تأقلمت معظم الشتلات في ظروف البيت الزجاجي. وبناء على النتائج فقد تم تطوير بروتوكول ناجح لإكثار *C. halicacabum* داخل الأنابيب. وهذا من شأنه أن يشجع على المزيد من العمل البحثي على *C. halicacabum* مثل البحث في مكوناته الطبية الفعالة وقوته المضادة للميكروبات.

الكلمات الدالة: C. halicacabum ، التكاثر داخل الأنابيب، الأردن، نبات طبي، التجذير، زراعة الأنسجة.

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