Journal of Medicinal and Industrial Plant Sciences (2026) 4 (1): 148- 155
P-ISSN: 2959 – 121X
E-ISSN: 3106 – 5406
DOI: https://doi.org/10.32894/MEDIP.26.4.1.10

In vitro propagation of *Helianthus annuus* L.

Zainab Hamid Abdul Rheem[1], Ibrahim Ali Ameen*[1], Susan Salahaddin Abdulla[2], Joned Khalaf Afnesh[2]

[1]*College of Medicinal and Industrial Plants, University of Kirkuk, Iraq.*
[2]*College of Veterinary Medicine, University of Kirkuk, Iraq*
[*] *Corresponding author: E-mail: ibrahimali@uokirkuk.edu.iq*

ABSTRACT
This study, conducted at the Central Laboratory for Plant Tissue Culture in Kirkuk, aimed to establish an efficient In Vitro protocol for Helianthus annuus L. by optimizing sterilization and micropropagation phases. The experimental framework, designed under a Completely Randomized Design (CRD) with ten replicates, first evaluated the efficacy of Sodium Hypochlorite (as commercial Clorox) at varying concentrations (0, 20, 40, and 60%). Results indicated that while the absence of the sterilizing agent led to total contamination, a 40% concentration significantly minimized the infection rate to 30% without compromising tissue integrity. Subsequently, the research investigated the morphogenic influence of the cytokinin BA (0, 1.0, 1.5, and 2.0 mg L^{-1}) on vegetative development. Statistical analysis revealed that the 2.0 mg L^{-1} BA treatment was the most effective for enhancing biomass and growth indicators, yielding the highest mean values for node number (3.250 per explant), leaf count (7.000 per shoot), and fresh and dry weights (0.862 g and 0.080 g, respectively). Interestingly, the control group exhibited the maximum shoot length (3.000 cm), suggesting a distinct response pattern to hormonal concentrations. These findings provide a standardized approach for the successful clonal propagation of sunflower industrial crops.

KEYWORDS: *Helianthus Annuus L., BA, Sodium Hypochlorite, propagation, In Vitro*.

Received: 22/02/2026; **Accepted**: 26/03/2026; **Available online:** 31/03/2026

اكثار نبات زهرة الشمس *Helianthus annuus L.* خارج الجسم الحي

زينب حامد عبدالرحيم[1]، ابراهيم علي امين[1]، سوزان صلاح الدين عبدالله[2]، جنيد خلف افنش[2]
كلية النباتات الطبية والصناعية، جامعة كركوك، كركوك، العراق.
كلية الطب البيطري، جامعة كركوك، كركوك، العراق

الملخص
نفذت التجربة في المختبر المركزي لزراعة الانسجة النباتية (قطاع خاص) في محافظة كركوك بهدف اختبار فعالية تأثير تراكيز مختلفة من هايبوكلورات الصوديوم في تعقيم بذور نبات ***Helianthus annuus L.*** وتحديد التركيز الامثل منها للقضاء على التلوث. بالاضافة الى دراسة تأثير تراكيز مختلفة من السايتوكاينين (**BA**) وتحديد التركيز الامثل منه في زيادة مؤشرات النمو الخضري و الوزن الرطب والجاف. طبقت التجارب النسيجية على وفق التصميم تام التعشية (**CRD**) بعشرة مكررات، اذ تم استعمال القاصر التجاري **Clorox** بتركيز(**0، 20، 40** و**60**%) لمدة ربع ساعة مع الرج المستمر بعد ذلك تمت دراسة تاثير تراكيز مختلفة من السايتوكاينين **BA**(0 ، 1.0 ، 1.5، 2.0) ملغم.لتر$^{-1}$. بينت نتائج التعقيم تلوث جميع البذور عند خلو محلول التعقيم من هايبوكلورات الصوديوم بينما اعطى التركيز 40 % من مادة التعقيم هيبوكلوريت الصوديوم اقل نسبة تلوث بلغت 30%. تأثير**BA** بالنسبة لمؤشرات النمو الخضري والوزن الرطب والجاف تفوق المعاملة بتركيز2.0 ملغم.لتر$^{-1}$ اذ اعطت اعلى متوسط لعدد العقد وعدد الاوراق والوزن الرطب و الجاف بلغ على التوالي **3.250**عقدة.جزء نباتي$^{-1}$، 7.000ورقة.فرع$^{-1}$، 0.862غم ،0.080 غم. بينما اعطت معاملة المقارنة اعلى معدل لطول الافرع بلغ 3.000 سم.
الكلمات المفتاحية: *زهرة الشمس* ، BA، هيبوكلورات الصوديوم، اكثار، **invitro**

INTRODUCTION

Industrial crops serve as a fundamental pillar for diverse manufacturing sectors, providing the essential raw materials required to drive economic growth and industrial productivity. Within this category, the sunflower (Helianthus annuus L.), a prominent member of the Asteraceae family, stands out as a globally significant industrial resource. The high concentration of unsaturated fatty acids, coupled with a rich profile of vitamins that inhibit oxidative degradation, distinguishes sunflower oil as one of the healthiest options for human consumption. Consequently, these nutritional merits have

solidified its position as a primary global commodity (Bilgen et al., 2018). In the local context of Iraq, agricultural data indicates a cultivated area of 2,381 dunums, with seed yields averaging approximately 1,183 kg per dunum (Directorate of Agricultural Statistics, 2022). However, to overcome the inherent constraints of traditional propagation, there has been a strategic shift toward advanced plant tissue culture methodologies. Micropropagation, in particular, has emerged as a pivotal technological application, facilitating large-scale commercial multiplication and ensuring the sustainability of high-quality plant stocks (Aldabbagh et al., 2024; Neumann et al, 2020). This technique has become extensively applied, particularly for the propagation of crop plants, ornamental plants, medicinal plants, fruit trees, and forest species that are difficult to propagate by conventional methods, as well as for producing plants outside their natural growing seasons (Wawrosch and Zotchev, 2021; Abdulhafiz et al., 2022). Based on the above, the present study aims to:

1. To evaluate the effectiveness of different concentrations of sodium hypochlorite in seed sterilization and to determine the optimal concentration for eliminating contamination.
2. To investigate the influence of various concentrations of the cytokinin BA and to identify the most suitable level for enhancing vegetative growth parameters, followed by measuring fresh and dry weight.

In a study conducted by AlIssawi et al. (2017) on sunflower plants, different concentrations of BA were used, and the mean number of shoots reached 1.76 shoots per explant at the concentration of 2.0 mg L^{-1}. In another study on plants belonging to the same family as sunflower (Asteraceae), Al-Hasani (2021) reported that the addition of BA at a concentration of 2.0 mg L^{-1} in Stevia resulted in the highest mean number of shoots and leaves, reaching 5.16 shoots per explant and 7.53 leaves, respectively.

MATERIALS AND METHODS

The experiment was carried out at the Central Laboratory for Plant Tissue Culture (private sector) in Kirkuk Province during the period from 1/10/2025 to 13/1/2026. Seeds of *Helianthus annuus* L. (cv. Vietnam) were used. The effectiveness of sodium hypochlorite in seed sterilization was evaluated using different concentrations of the commercial bleach Clorox containing 6% active ingredient (w/v). The solution was diluted to obtain sterilizing solutions with concentrations of 0, 20, 40, and 60% sodium hypochlorite. A few drops of the surfactant Tween-20 were added to each concentration to reduce the surface tension of the sterilizing solutions. The seeds were treated with the sterilizing solutions for 15 minutes and subsequently rinsed three times with sterile distilled water. To optimize surface sterilization, initial trials compared 40% and 60% sodium hypochlorite concentrations. While both treatments successfully reduced contamination to a 30% threshold, the 60% concentration induced significant phytotoxicity, leading to observable tissue degradation in the emerging seedlings. Based on these findings, the 40% concentration was standardized for all subsequent experiments to

maintain explant health. The incidence of microbial contamination was recorded ten days post-inoculation as a percentage of the total cultured seeds. Following the initiation phase, the morphogenic effect of Benzyladenine (BA) was investigated at concentrations of 0, 1.0, 1.5, and 2.0 mg L^{-1}. For each treatment, ten independent replicates were established, where nodal segments were precisely excised and transferred to the multiplication media at a density of one explant per replicate. The cultures were incubated in the growth room under a light intensity of 1000 lux and a 16 h light / 8 h dark photoperiod. After the incubation period, vegetative growth parameters including number of nodes, number of leaves, shoot length, as well as fresh and dry weight were measured.

RESULTS AND DISCUSSION

Effect of surface sterilization using sodium hypochlorite solution on seeds of *Helianthus annuus* L. after 10 days of in vitro culture

The results presented in Table 1 demonstrate the effectiveness of different concentrations of sodium hypochlorite (NaOCl) in reducing contamination of *Helianthus annuus* L. seeds. The lowest contamination percentage was observed at concentrations of 40% and 60%, reaching 30% for both treatments, respectively. In contrast, complete contamination was recorded in the control treatment where sodium hypochlorite (NaOCl) was not applied.

Table 1. Effect of surface sterilization with sodium hypochlorite (NaOCl) solution on seeds of *Helianthus annuus* L. after 10 days of in vitro culture.

Percentage of seed contamination	NaOCl concentrations (%)
100	0
50	20
30	40
30	60

It was observed that increasing the concentration of sodium hypochlorite to 60% for 15 minutes led to delayed seed germination or complete inhibition of germination, in addition to the appearance of abnormal seedlings. In contrast, the concentration of 40% sodium hypochlorite resulted in the lowest contamination rate, reaching 30%, without negatively affecting germination. This effect may be attributed to the toxic influence of high concentrations of NaOCl on plant tissues. Similarly, Islam et al. (2021) reported that the use of sodium hypochlorite at a concentration of 40% in *Helianthus annuus* L. was effective in obtaining contamination-free seedlings compared with the control treatment.

Effect of BA on the mean number of nodes of *Helianthus annuus* L. in vitro after four weeks of culture

The results shown in Table 2 indicate that the addition of BA to the multiplication media of *Helianthus annuus* L. resulted in a significant increase in the average number of formed nodes, reaching its maximum at the concentration of 2.0 mg L^{-1}, which produced the highest mean number of nodes, 3.250 nodes per explant, compared with the control treatment that recorded 1.750 nodes per explant.

Table 2. Effect of BA on the mean number of nodes of *Helianthus annuus* L. in vitro after four weeks of culture.

BA (mg L^{-1})	Number of nodes
0	1.750
1.0	2.000
1.5	2.500
2.0	3.250
LSD 0.05	1.133

Effect of BA on the mean number of leaves of *Helianthus annuus* L. in vitro after four weeks of culture

The results presented in Table 3 show that the concentration of 2.0 mg L^{-1} BA was superior to the other concentrations in increasing the mean number of leaves compared with the control treatment. The highest mean number of leaves reached 7.00 leaves per shoot, whereas the lowest value was recorded in the control treatment, which produced 4.00 leaves per shoot.

Table 3. Effect of BA on the mean number of leaves of *Helianthus annuus* L. in vitro after four weeks of culture.

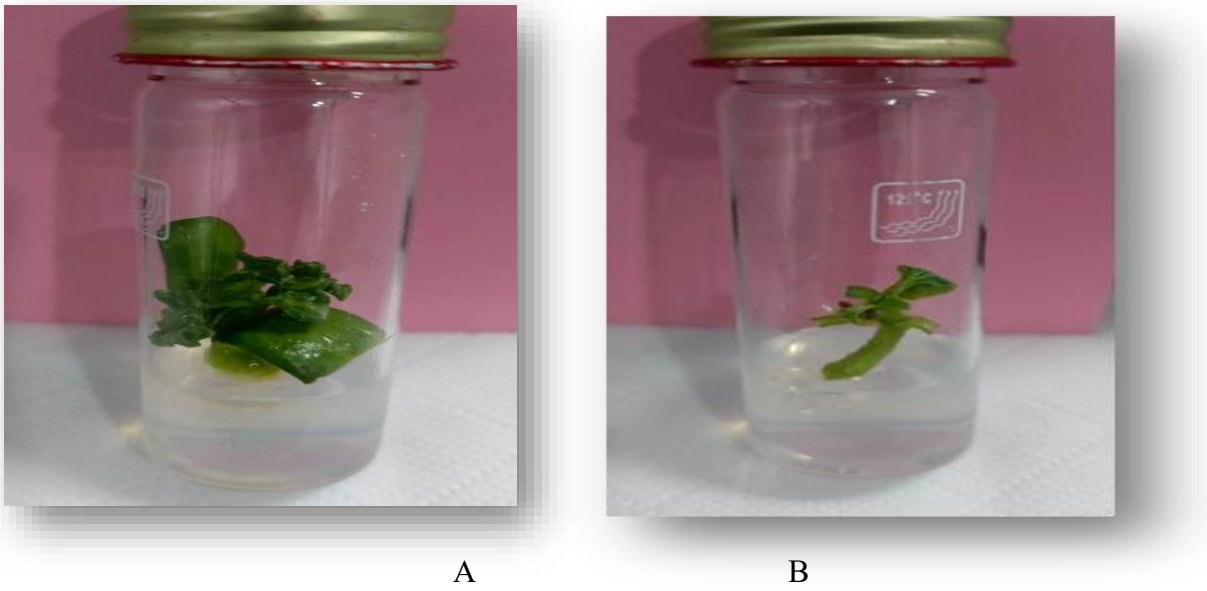
BA (mg L^{-1})	Number of leaves
0	4.000
1.0	4.250
1.5	5.000
2.0	7.000
LSD 0.05	1.925

Effect of BA on the mean shoot length of *Helianthus annuus* L. in vitro after four weeks of culture

The results presented in Table 4 indicate that the addition of different concentrations of BA significantly reduced the mean shoot length of *Helianthus annuus* L. in vitro. The control treatment recorded the highest mean shoot length, reaching 3.750 cm. Shoot length then decreased significantly with increasing BA concentrations, reaching the lowest mean value at the concentration of 2.0 mg.L^{-1}.

Table 4. Effect of BA on the mean shoot length of *Helianthus annuus* L. in vitro after four weeks of culture.

BA (mg L^{-1})	Plant height (cm)
0	3.750
1.0	3.625
1.5	3.500
2.0	3.000
LSD 0.05	1.504



A B

Figure 1. (A) Explant grown on MS medium without growth regulators, **(B)** explant growing on MS culture medium supplemented with 2.0 mg L^{-1} BA.

It can be inferred from the results presented in Tables 2 and 3 that BA was effective in increasing the mean number of nodes and leaves and in promoting shoot multiplication. This effect may be attributed to the molecular structure of this cytokinin and the number of double bonds present in its side chain, as it contains three double bonds, in addition to the presence of a benzyl ring, which has made BA one of the most widely used cytokinins in plant propagation (Abdul, 1987). Moreover, BA is considered a stable compound that does not easily degrade and has a high efficiency in breaking apical dominance. It also contributes to the enlargement of the vascular tissues, including xylem vessels and phloem sieve tubes, prevents chlorophyll degradation, and stimulates cell division as well as the synthesis of nucleic acids (Mok et al., 2000; Schmulling, 2004). These results are consistent with the findings reported by AlIssawi et al. (2017) and Al-Hasani (2021).

It was also observed that increasing the concentration of cytokinin in the culture medium up to the optimal level led to an increase in the mean number of nodes. This may be attributed to the fact that

higher cytokinin concentrations in the culture medium enhance the response rate until reaching the optimal condition, which occurs when hormonal balance is achieved, resulting in the maximum growth response of the cultured explants. In addition, cytokinins play an important role in stimulating cell division and differentiation as well as promoting the growth of axillary buds (Dellolio, 2007).

The results of the present study also demonstrated the importance of cytokinins in increasing shoot length. As shown in Table 4, the treatment with 1.0 mg L^{-1} BA produced the highest mean shoot length, reaching 3.625 cm. This effect may be attributed to the role of cytokinins in stimulating cell division and promoting cell growth (Al-Khafaji, 2014).

Effect of BA on the mean fresh weight (g) of shoots of *Helianthus annuus* L. in vitro

The results presented in Table 5 indicate that higher concentrations of BA added to the culture medium resulted in a significant increase in the mean fresh weight of shoots. Data analysis revealed that the 2.0 mg L^{-1} concentration exerted the most profound influence on biomass accumulation, significantly outperforming all other treatments. This concentration achieved a peak shoot fresh weight of 0.862 g, representing the maximum growth response observed in the study. Conversely, the 1.0 mg L^{-1} treatment resulted in the minimum incremental gain, recording the lowest fresh weight at 0.777 g. These findings underscore a clear dose-dependent relationship between BA levels and the vegetative development of the explants.

Table 5. Effect of BA on the mean fresh weight of shoots of *Helianthus annuus* L. in vitro after four weeks of culture.

BA (mg L^{-1})	Fresh weight
0	0.682
1.0	0.777
1.5	0.810
2.0	0.862
LSD 0.05	0.409

Effect of BA on the mean dry weight (g) of shoots of *Helianthus annuus* L. in vitro

The results presented in Table 6 indicate that higher concentrations of BA added to the culture medium resulted in a significant increase in the mean dry weight of shoots. The concentration of 2.0 mg L^{-1} showed the greatest effect, significantly exceeding the other treatments and producing the highest dry weight of shoots, reaching 0.080 g, compared with the control treatment, which recorded the lowest value of 0.070 g.

Table 6. Effect of BA on the mean dry weight of shoots of *Helianthus annuus* L. in vitro after four weeks of culture.

BA (mg L^{-1})	Dry weight
0	0.059
1.0	0.070
1.5	0.071
2.0	0.080
LSD 0.05	0.004

The increase in fresh and dry weight of shoots of *Helianthus annuus* L. reflects changes in the cellular components of the shoots as influenced by their growth in the culture medium, which primarily depends on the plant growth regulators added to the medium. In general, cell division in the shoots is accompanied by an increase in essential cellular constituents required for sustaining growth and division, such as carbohydrates, proteins, and amino acids. These internal biochemical changes promote cell division and enlargement, which was clearly observed in the treatment supplemented with 2.0 mg L^{-1} BA.

CONCLUSIONS

1. The absence of sodium hypochlorite (NaOCl) during sterilization resulted in severe contamination of sunflower explants (stem nodes) cultured in vitro, whereas higher concentrations caused damage to the plant tissues and resulted in their death.
2. The cytokinin BA was effective in improving vegetative growth traits as well as increasing the fresh and dry weight of sunflower plants.

RECOMMENDATIONS

1. Further development of plant tissue culture techniques for the in vitro propagation of sunflower through studies focusing on shoot and leaf production, as well as improving the success rate of plant acclimatization under external conditions.
2. The use of sodium hypochlorite (NaOCl) at a concentration of 40% is recommended for effective contamination control.

The cytokinin BA at a concentration of 2.0 mg L^{-1} is recommended to obtain the highest response in vegetative growth parameters and fresh and dry weight of sunflower plants.

REFERENCES

Abdul, K. S. (1987). *Plant growth regulators (Part 1).* Salahaddin University, Ministry of Higher Education and Scientific Research, Republic of Iraq.

Abdulhafiz, F., Mohammed, A., Reduan, M. F. H., Kari, Z. A., Wei, L. S., & Goh, K. W. (2022).

Plant cell culture technologies: A promising alternative to produce high-value secondary metabolites. *Arabian Journal of Chemistry, 15*(11), 104161. https://doi.org/10.1016/j.arabjc.2022.104161

Aldabbagh, F. M. K., Al-Zaidi, I. H. M., & Alshamari, M. A. K. (2024). Micropropagation and assessment of genetic fidelity of regenerates by RAPD markers of *Solanum nigrum*. *Iraqi Journal of Agricultural Sciences, 55*(1), 432–439. https://doi.org/10.36103/tnx6m684

Al-Hasany, Z. H. A. (2021). *The potential of increasing sweetening compounds and studying molecular markers in EMS mutated tissue cultures of Stevia rebaudiana Bertoni plants* (Master's thesis, University of Baghdad, College of Agricultural Engineering Sciences).

AlIssawi, A. Y. H., Abbood, H. A. N., Jaber, T. F., Noor, W. K., Kadhim, D. A. H., Hussein, S. H., Habeeb, S. A. K., & Alabbasi, I. J. (2017). Shoot propagation from three sunflower (*Helianthus annuus* L.) genotypes via callus induction *in vitro*. *International Journal of Advanced Research in Medical & Pharmaceutical Sciences, 2*(12).

Al-Khafaji, Z. M., & Abu Al-Maali, H. M. (2013). *Polymerase chain reactions and primer design.* University of Baghdad, Ministry of Higher Education and Scientific Research, Republic of Iraq.

Bilgen, B. B., Evci, G., & Kaya, Y. (2018). Comparison of different DNA markers for selection of high oleic type sunflower genotypes. In *International Agricultural, Biological & Life Science Conference* (pp. 140–145).

Central Statistical Organization. (2022). *Statistical directorate data report.* Ministry of Planning, Republic of Iraq.

Dell'Olio, R. (2007). Cytokinins determine *Arabidopsis* root meristem size by controlling cell differentiation. *Current Biology, 17*, 678–682.

Islam, K., Ahmed, T., & Sharmin, T. (2021). In vitro growth of sunflower (*Helianthus annuus*) via direct organogenesis. *American Journal of Pure and Applied Biosciences, 3*, 60–64.

Mok, M. C., Martin, R. C., & Mok, D. W. (2000). Cytokinins: Biosynthesis, metabolism and perception. *In Vitro Cellular & Developmental Biology - Plant, 36*(2), 102–107.

Neumann, K. H., Kumar, A., & Imani, J. (2020). *Plant cell and tissue culture: A tool in biotechnology.* Springer. https://doi.org/10.1007/978-3-030-49098-0

Schmülling, T. (2004). Cytokinins. In *Encyclopedia of Biological Chemistry*. Elsevier.

Wawrosch, C., & Zotchev, S. B. (2021). Production of bioactive plant secondary metabolites through in vitro technologies—Status and outlook. *Applied Microbiology and Biotechnology, 105*(18), 6649–6668. https://doi.org/10.1007/s00253-021-11539-w