

Influence of Auxin, Cytokinin, and Amino Acid Interaction on Fresh and Dry Weight of *Moringa Oleifera* L. Callus: A Biotechnological Approach

Hassan Aziz Fadil Alzaydi¹, Basheer Abdulhamza Mohammed Alalwani¹

¹ University of Babylon, College of Science, Department of Biology, Iraq

ABSTRACT

Background: *Moringa oleifera* L. is a medicinally significant plant with bioactive compounds that have potential applications in pharmacology and pathology. Plant tissue culture techniques, particularly callus induction, provide a controlled system for studying secondary metabolite production and optimizing growth parameters. **Objective:** This study aimed to evaluate the effect of auxin (2,4-Dichlorophenoxyacetic acid - 2,4-D) and cytokinin (Benzyl adenine - BA) interactions, supplemented with amino acids (Tryptophan and Phenylalanine), on fresh and dry weight accumulation in *Moringa oleifera* L. callus cultures. **Methods:** Callus induction was performed using Murashige and Skoog (MS) medium supplemented with different combinations of 2,4-D (1, 2 mg/L), BA (0, 2 mg/L), Tryptophan (5, 10, 25 mg/L), and Phenylalanine (50, 100, 200 mg/L). Fresh and dry weights of the callus were recorded and statistically analyzed using Duncan's multiple range test. **Results:** Among the tested treatments, 2,4-D (1 mg/L) + Tryptophan (5 mg/L) demonstrated the highest fresh and dry weight accumulation in callus cultures, suggesting an optimal hormonal and amino acid interaction. Additionally, the combination of 2,4-D (2 mg/L) + BA (2 mg/L) + Phenylalanine (200 mg/L) showed a significant increase in both parameters compared to other treatments. **Conclusion:** The study confirms that the interaction between auxins, cytokinins, and amino acids plays a crucial role in enhancing callus biomass in *Moringa oleifera* L. The findings have potential applications in pharmaceutical research, particularly for optimizing secondary metabolite production in medicinal plants using plant tissue culture techniques.

Keywords: 2,4- Dichlorophenoxyiacetic Acid, Benzyl Adanine, Tryptophan, Phenyl Alalnine, *Moringa Oleifera*, Callus

INTRODUCTION

Moringa oleifera is a drought-resistant and fast-growing tree of the family Moringaceae, native in South Asia's tropical or subtropical regions. It is edible in all parts of *Moringa* [1]. *Moringa oleifera* is classified into the following: Kingdom: Plantae, Division: Magnoliophyta, Class: Magnoliopsida, Order: Brassicales, Family: Moringaceae, Genus: *Moringa*, Species: *M. oleifera* [2]. *Moring* is an old plant commonly known for different horseradish trees or drumstick trees [3]. *Moringa oleifera* is an important food commodity. The leaves contain more calcium than milk and contain more vitamin A compared to carrots [4]. Tissue culture techniques encourage the biosynthesis and growth of many secondary metabolic compounds in plant tissues, so they have been considered the most promising technologies in the pharmaceutical industry [5]. Tissue culture has also been used to isolate cell lines from callus cultures that produce typical levels of secondary metabolites in several plants, including *Moringa oleifera* [4]. Using various explants of plants in tissue culture, such as leaves, cotyledon, seed, shoots, and roots [6]. There are many motivators that can be abiotic, like temperature, saline, and salicylic acid, which were applied to increase the biosynthesis of many metabolic compounds in the in vitro cultures of *M. oleifera*, including phenolic acids and flavonoid compounds [7]. Tissue culture techniques are important in studying plant physiology, biochemistry, and pathology [8]. Amino acids are organic compounds that contain two active functional groups: the amine group - NH₂ - and the carboxyl group - COOH -, or they are small repeating units linked together to form proteins, which are complexes with high molecular weights, as well as peptides, which are simpler in complexity, and are among the most important sources of nitrogen. It has a role in controlling the growth and development of cells and the formation of

somatic embryos in plant tissue cultures [9]. Amino acids are different depending on the presence of the R group, as they all consist of a carbon atom (alpha) linked to four different groups: the amine, the carboxyl, the hydrogen atom, and the terminal (R group), except for glycine, which has a symmetrical carbon atom (H: R). Amino acids have several functions within biological cells, such as enzymes, which are proteins involved in biological reactions, and also their importance as antibiotics, which are specialized protein molecules to support the immune system against foreign antigens [10].

MATERIALS AND METHODS

Collection and Sterilization of Seeds

Seeds were collected from nurseries in the Babylon Governorate and diagnosed in the laboratories of the College of Science/ University of Babylon. The seeds were washed and immersed in water to get rid of the dirt and dust stuck in them, and they were washed with bubble gum, and they were transferred to the stratified air cabin, where they were sterilized with 7% sodium hypochlorate. They were washed with distilled water three times, each time for a minute, and they were washed once for a minute with 70% ethyl alcohol with continuous stirring, and they were washed again with distilled water three times, one minute each time, to remove any remaining alcohol [11].

Preparation of Growth Regulator Solutions

A suitable basic solution for all plant growth regulators used in the experiments was prepared by dissolving 50 mg of 2,4-D in 1 ml of absolute ethyl alcohol to ensure the full dissolving and complete volume to 50 ml with distilled water to become our fundamental

solution concentration of 1 mg/ml, while benzyl adenine (BA) was prepared by dissolving 50 mg of BA in 1 ml of HCl to ensure the full dissolving and complete volume to 50 ml with distilled water to become our fundamental solution concentration of 1 mg/ml.

Culture Medium

Moringa seeds were used to stimulate callus growth, and growth medium (MS) was used with the addition of some growth hormones (2,4-D) and (BA) in different concentrations. Prepare the growth medium (MS) by adding 4.44 g per liter of distilled water, sugar in the amount of 30 g, and growth regulatory hormone in different concentrations. The solution was calibrated to 5.8 (PH) with 0.1 N NaOH or HCl before that. Agar was added in the amount of 7 g and placed on a device. Heating. Until the color of the solution becomes transparent, distribute the solution into special tubes, 10 for each tube, then transfer it to the autoclave for 15 minutes under a pressure of 15 pounds/m² and a temperature of 121°C. It was transferred to the air chamber to grow the pre-prepared seeds in tubes, after which it was incubated with varying periods between light and darkness daily, and the temperature was 25 + 2 degrees Celsius.

Results and Discussion

Table (1) shows the effect of 2 mg/L concentrations of the hormone 2,4-D on callus induction in the *Moringa oleifera* plant and the effect of adding amino acids by measuring the wet weight. The best significant response was the treatment of the hormone 2,4 D1 (ph) 200, which reached (1.281) mg, while the other treatments were lower significantly, reaching (1.095-1.208) mg for each of 50 and 100 mg/L of phenyl alanine acid, respectively. Treatment of (2,4 D1 (typ) 5) gave the best significant response in increasing wet weight, reaching (1.919) mg, while the effect of the other treatments was variable (1.423,1.124) mg for each of (10,25) mg/L for the amino acid tryptophan. We also note that the (2,4 D2 (ph) 50) treatment gave the best increase in wet weight, reaching (1.730) mg compared with treatments (2,4 D2 (ph) 100, 2,4 D2 (ph) 200), which reached (1.331, 1.271) mg, respectively. The 2,4 D2 (typ) 25 treatment gave the highest

value (1.612) mg for the wet weight, while the other treatments were (1.528–1.436) mg for 2,4 D2 (typ) 5 and 2,4 D2 (typ) 10, respectively. Table (2) shows that the 2,4 D1 (ph) 200 treatment gave the best dry weight of callus in the *Moringa oleifera* L. plant compared to the of the treatments, 2,4 D1 (ph) 50 and 2,4 D1 (ph) 100, where it reached (0.8, 0.86) mg, while we notice that the treatment of 2,4 D1 typ 5 had an effect in increasing the dry weight significantly (1.45) mg, while the other treatments amounted to (0.8, 0.83) mg for each of 2,4 D1 (typ) 10 and 2,4 D1 (typ) 25 respectively. The 2,4 D2 (ph) 50 treatment gave the highest dry weight of (1.33) mg, while the 2,4 D2 (ph) 100 and 2,4 D2 (ph) 200 treatments had a different effect (1.05, 1) mg, respectively. The highest value was in treatment 2,4 D2 (typ) 25, which amounted to (1.19) mg, compared to the other treatments, 2,4 D2 (typ) 5, and 2,4 D2 (typ) 10, which amounted to (1.15, 1.13) mg, respectively. From Table (3), it can be noticed that using a combination between the hormones auxin and cytokinin, 2,4 D1+ BA 0.5 (ph) 200 gave the highest significant value for fresh weight, which amounted to (1.145) mg, compared to the other treatments. The highest value in fresh weight was also recorded in the treatment 2,4 D1+ BA 0.5 (typ) 10, where it reached (1.1) mg, while the values were (1.008, 1.075) mg in 2,4 D1+ BA 0.5 (typ) 5, 2, 4, and 4 D1+ BA 0.5 (typ) 25 respectively. The 2,4 D2 + BA 2 (ph) 200 treatment had an effect on increasing the fresh weight (1.333) mg, while the other treatments were less effective in their effect on the fresh weight (1.262, 1.17) mg for each of 2,4 D2 + BA 2 (ph) 50, 2,4 D2 + BA 2 (ph) 100, respectively. The highest value in the treatment 2,4 D2+ BA 2 (typ) 25 was (1.132), while it was (1.005, 1.048) mg for 2,4 D2+ BA 2 (typ) 5, 2,4 D2+ BA 2 (typ) 10. Table (4) shows the effect of hormones, where treatment 2,4 D1+ BA 0.5 (ph) 200 gave the highest value in dry weight (0.88) mg compared to the other treatments. The treatment 2,4 D1+ BA 0.5 (typ) 10 affected the increase in dry weight, reaching (0.92) mg. While the treatment 2,4 D2+ BA 2 (ph) 200 affected the weight gain and reached (1.18) mg compared to the other treatments 2,4 D2 + BA 2 (ph) 50, 2,4 D2+ BA 2 (ph) 100, which gave (1.01, 1) mg, respectively, 2,4 D2+ BA 2 (typ) 25 was the best in increasing dry weight (1) mg.

Table 1: Effect of (1 mg/L) of 2,4-D Combined with Tryptophan and Phenylalanine on Wet Weight of *Moringa Oleifera* L. Callus (mg)

No	Name (ppm)	Wet weight
1	2,4 D1 (ph) 50	1.095 d
2	2,4 D1 (ph) 100	1.208 d
3	2,4 D1 (ph) 200	1.281 bc
4	2,4 D1 (typ) 5	1.919 a
5	2,4 D1 (typ) 10	1.423 bc
6	2,4 D1 (typ) 25	1.124 d
7	2,4 D2 (ph) 50	1.730 bc
8	2,4 D2 (ph) 100	1.331 bc
9	2,4 D2 (ph) 200	1.271 bc
10	2,4 D2 (typ) 5	1.530 bc
11	2,4 D2 (typ) 10	1.436 bc
12	2,4 D2 (typ) 25	1.615 bc

Table 2: Effect of (1) mg/L of 2,4-D Combined with Tryptophan and Phenylalanine on Dry Weight of *Moringa Oleifera* L. Callus (mg)

No	Name (ppm)	Dry weight
1	2,4 D1 (ph) 50	0.80 e
2	2,4 D1 (ph) 100	0.86 de
3	2,4 D1 (ph) 200	0.93 de
4	2,4 D1 (typ) 5	1.45 a
5	2,4 D1 (typ) 10	0.80 e
6	2,4 D1 (typ) 25	0.83 de
7	2,4 D2 (ph) 50	1.33 bc
8	2,4 D2 (ph) 100	1.11 bcde
9	2,4 D2 (ph) 200	1.00 cde
10	2,4 D2 (typ) 5	1.15 bcde
11	2,4 D2 (typ) 10	1.13 bcde
12	2,4 D2 (typ) 25	1.19 bcd

Table 3: Effect of Hormone 2,4-D and BA combined with Tryptophan and Phenylalanine on Wet Weight of *Moringa Oleifera* L. Callus (mg).

No	Name (ppm)	Wet weight
1	2,4 D1+ BA0.5 (ph) 50	1.079 b
2	2,4 D1+ BA0.5 (ph) 100	1.123 b
3	2,4 D1+ BA0.5 (ph) 200	1.145 b
4	2,4 D1+ BA0.5 (typ) 5	1.008 b
5	2,4 D1+ BA0.5 (typ) 10	1.100 b
6	2,4 D1+ BA0.5 (typ) 25	1.075 b
7	2,4 D2+ BA 2 (ph) 50	1.262 b
8	2,4 D2+ BA 2 (ph) 100	1.170 b
9	2,4 D2+ BA 2 (ph) 200	1.333 a
10	2,4 D2+ BA 2 (typ) 5	1.005 b
11	2,4 D2+ BA 2 (typ) 10	1.048 b
12	2,4 D2+ BA 2 (typ) 25	1.132 b

Table 4: Effect of Hormone 2,4-D and BA combined with Tryptophan and Phenylalanine on Dry Weight of *Moringa Oleifera* L. Callus (mg)

No	Name (ppm)	Dry weight
1	2,4 D1+ BA0.5 (ph) 50	0.80 b
2	2,4 D1+ BA0.5 (ph) 100	0.84 b
3	2,4 D1+ BA0.5 (ph) 200	0.88 b
4	2,4 D1+ BA0.5 (typ) 5	0.79 b
5	2,4 D1+ BA0.5 (typ) 10	0.92 b
6	2,4 D1+ BA0.5 (typ) 25	0.80 b
7	2,4 D2+ BA 2 (ph) 50	1.01 b
8	2,4 D2+ BA 2 (ph) 100	1.00 b
9	2,4 D2+ BA 2 (ph) 200	1.41 a
10	2,4 D2+ BA 2 (typ) 5	0.85 b
11	2,4 D2+ BA 2 (typ) 10	0.95 b
12	2,4 D2+ BA 2 (typ) 25	1.00 b

It can be noted from the results of the tables above that the effect of amino acid interaction with the effect of plant hormones on fresh and dry weights is significant, and this is confirmed by the results of [12], where it was stated that the use of cytokinins such as BA leads to increased absorption of nutrients in plant tissue and thus an increase in dry weight.

Conclusion

The success of callus induction depends on the genetic makeup of the plant used as a plant [12]. The presence of the hormone auxin and its stimulation by the hormone 2,4-D is the main reason for the decrease in fresh weight of callus at higher concentrations of 2,4-D in the callus growth medium. The production of ethylene gas, which reduces cell division and growth rate [13], or the amounts of auxin that enter the cells as well as the rate of auxin metabolism within the tissues, in addition to the amount of internal auxin that the cells lose, To average [14]. It is indicated that 2,4-D was better than other growth regulators such as NAA, IBA, and IAA in stimulating callus tissue. The results of this study indicated that a concentration of 2,4-D at a concentration of 2 mg/L was considered the best concentration for initiating callus tissue. Callus infection of *Trigonella* seeds, which was used in all treatments [15]. It was pointed out that the hormone 2,4-D has a stimulating effect on the number of calluses cells and also has an effect on increasing the size of the cells as a result of increasing the water content in the cells. Based on these results, the hormone 2,4-D was used. Which is used in the growth medium to induce callus from *Targonella* seeds to obtain the best weight of the induced callus and is used in the accumulation stage [16]. These results are consistent with the results of [17], which reported that the medium supplemented with the hormone 2,4-D at a concentration of 2 mg/L was better at reducing

the fresh weight of callus induced by *Trigonella* compared to other hormones such as (NAA, IBA, and IAA). Based on the fresh and dry weight values of callus in this study, the medium supplemented with (2 mg/L) 2,4-D was used in subsequent experiments for the accumulation phase. The researchers indicated that the MS medium was the best. Medium to induce callus tissue [2, 18]. Tryptophan has a well-known role: it plays an indirect role for amino acids in growth through its effect on auxin synthesis. It has been reported that alternative routes for supplying tryptophan to economically important chemical components could be some plant tissues, IAA has been formed, and interestingly, via both tryptophan and auxin treatments [19]. The utilization of amino acids as a precursor of plant growth hormone is one way to limit the impact of water stress on plant development and productivity. A typical precursor of the plant hormone auxin is tryptophan, which influences the physiological processes of plants after absorption, either directly or indirectly, after converting into auxins (IAA). Tryptophan is an amazing amino acid, it might go about as an osmolyte, particle transport controller adjusts stomatal opening and detoxifies the harmful effects of heavy metals. Besides, the tryptophan pathway assumes a protective role in plants [16, 20]. Abiotic factors, such as amino acids, when added to the medium at a low concentration, lead to a decrease in fresh and dry weight. It is explained that high concentrations lead to variations in water relations and increased absorption of nutrients into the plant tissue [17], and thus may reduce or increase the growth of callus cells [18]. It may also be that adding the amino acid Phy to the nutrient medium causes a decrease in callus growth because it is the first precursor to the synthesis of SA, which affects cell growth [20]. The fresh and dry weight of callus increases in high concentrations because it is considered the main precursor for many active compounds and hormones.

REFERENCES

- [1] M. Awasthi *et al.*, "Comparative assessment of ethnobotany and antibacterial activity of *Moringa oleifera* Lam. in Nepal," *Ethnobotany Research and Applications*, vol. 25, pp. 1-13, 2023.
- [2] S. A. Sarode, Y. N. Sonawane, R. K. Suralkar, D. D. Kumbhar, P. P. Warade, and P. R. Patil, "Moringa Oleifera: Phytochemistry, pharmacology," *GSC Biological and Pharmaceutical Sciences*, vol. 24, no. 3, pp. 041-055, 2023.
- [3] S. Arshad, B. Sajjad, A. Aftab, Z. Yousaf, and M. O. Alotaibi, "Moringa. In Essentials of Medicinal and Aromatic Crops (pp. 1063-1089). Cham," *Springer International Publishing*, vol. 82, no. 9, pp. 2379-2386, 2023.
- [4] L. Zanella *et al.*, "Induction of antioxidant metabolites in *Moringa oleifera* callus by abiotic stresses," *Journal of natural products*, vol. 82, no. 9, pp. 2379-2386, 2019, doi: 10.1021/acs.jnatprod.8b00801.
- [5] C. A. Espinosa-Leal, C. A. Puente-Garza, and S. García-Lara, "In vitro plant tissue culture: means for production of biological active compounds," *Planta*, vol. 248, pp. 1-18, 2018.
- [6] O. J. Sexton, J. E. Bramble, I. L. Heisler, C. A. Phillips, and D. L. Cox, "Eggshell composition of squamate reptiles: relationship between eggshell permeability and amino acid distribution," *Journal of chemical ecology*, vol. 31, pp. 2391-2401, 2005.
- [7] E. Ortega-Hernández, M. Antunes-Ricardo, and D. A. Jacobo-Velázquez, "Improving the health-benefits of kales (*Brassica oleracea* L. var. acephala DC) through the application of controlled abiotic stresses: A Review," *Plants*, vol. 10, no. 12, p. 2629, 2021.
- [8] S. Rahayu, I. Roostika, and N. Bermawie, "The effect of types and concentrations of auxins on callus induction of *Centella asiatica*," *Nusantara Bioscience*, vol. 8, no. 2, pp. 283-287, 2016.
- [9] W. Zevenboom, A. Bij de Vaate, and L. R. Mur, "Assessment of factors limiting growth rate of *Oscillatoria agardhii* in hypertrophic Lake Wolderwijd, 1978, by use of physiological indicators 1," *Limnology and Oceanography*, vol. 27, no. 1, pp. 39-52, 1982.
- [10] K. Al-Hattmy, "Comparative study of atropine alkaloids production in vivo and in vitro of two type of *Datura*: *Datura metel* Linn and *Datura innoxia* Mill," Thesis Ph. D, College of Science, University of Kufa, Iraq, 2006.
- [11] N. H. Ali and B. A. Mohammed Alalwani, "EFFECT OF 2, 4-D, TRYPTOPHAN AND POLYETHYLENE GLYCOL ON GROWTH OF *TRIGONELLA FOENUM-GRACUM* L CALLUS," *Biochemical & Cellular Archives*, vol. 21, no. 1, 2021.
- [12] T. A. Altabtabai, B. A. Alalwani, and I. S. Abbas, "HPLC analysis of the trigonelline and diosgenin in the callus of fenugreek (*Trigonella foenum graecum* L.) seeds," *Plant Archives*, vol. 19, no. 2, pp. 2046-2050, 2019.
- [13] R. Casanova-Sáez, E. Mateo-Bonmatí, and K. Ljung, "Auxin metabolism in plants," *Cold Spring Harbor Perspectives in Biology*, vol. 13, no. 3, p. a039867, 2021.
- [14] K. Jamwal, S. Bhattacharya, and S. Puri, "Plant growth regulator mediated consequences of secondary metabolites in medicinal plants," *Journal of applied research on medicinal and aromatic plants*, vol. 9, pp. 26-38, 2018.
- [15] L. A. Erland and P. Saxena, "Auxin driven indoleamine biosynthesis and the role of tryptophan as an inductive signal in *Hypericum perforatum* (L.)," *PLoS One*, vol. 14, no. 10, p. e0223878, 2019.
- [16] Y. A. Khalifa, A. El-Naem, F. Gamal, and M. A. Mahmoud, "Effect of tryptophan and ascorbic acid on yield and some chemical constituents of lupine (*Lupinus termis* L.) plants," *Egyptian Journal of Agronomy*, vol. 42, no. 1, pp. 47-61, 2020.
- [17] M. Saker, M. Rady, and S. Ghanem, "Elicitation of tropane alkaloids in suspension cultures of *Hyoscyamus*, *Datura* and *Atropa* by osmotic stress," 1997.
- [18] R. Radman, T. Saez, C. Bucke, and T. Keshavarz, "Elicitation of plants and microbial cell systems," *Biotechnology and applied biochemistry*, vol. 37, no. 1, pp. 91-102, 2003.
- [19] N. J. Jasim Al-Saedi, L. K. Jawad Al-Ameri, and S. A. Mahdi, "Medicinal importance of *Atropa belladonna* plant (tropane alkaloids)," *Journal of Kirkuk University for Agricultural Sciences*, vol. 14, no. 3, 2023.
- [20] S. Hayat, B. Ali, and A. Ahmad, "Salicylic acid: biosynthesis, metabolism and physiological role in plants," *Salicylic acid: A plant hormone*, pp. 1-14, 2007.



This is an open-access article distributed under the terms of the Creative Commons Attribution License.