

Influence of Tryptophan and Phenylalanine on Antioxidant Enzyme Activity in *Moringa Oleifera* Callus Culture

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ABSTRACT

Background: *Moringa oleifera* is a medicinal plant known for its high antioxidant capacity, largely attributed to its enzymatic defense system. The manipulation of plant tissue culture conditions, including amino acid supplementation, can influence the production of antioxidant enzymes, which have potential biomedical and pharmaceutical applications. **Objective:** This study aimed to evaluate the effect of tryptophan and phenylalanine supplementation on the activity of key antioxidant enzymes—catalase (CAT), superoxide dismutase (SOD), and peroxidase (POD)—in *Moringa oleifera* callus cultures. **Methods:** The experiment was conducted at the College of Science, University of Babylon, using Murashige and Skoog (MS) culture medium supplemented with different concentrations of tryptophan (5, 10, 25 mM) and phenylalanine (50, 100, 200 ppm). Growth regulators, including 2,4-Dichlorophenoxyacetic acid (2,4-D) and Benzyl Adenine (BA), were also added at concentrations of 0.5, 1, and 2 mg/L. The enzymatic activity of CAT, SOD, and POD was measured spectrophotometrically to determine the impact of these treatments on the plant's antioxidant defense system. **Results:** The results demonstrated a significant influence of amino acid supplementation on antioxidant enzyme activity. Catalase and SOD activity increased significantly in the presence of tryptophan (2,4-D 1 mg/L + BA 0.5 mg/L + Try 10 mM) and phenylalanine (2,4-D 1 mg/L + BA 0.5 mg/L + Ph 50 ppm). Peroxidase activity showed the highest increase at tryptophan (2,4-D 2 mg/L + Try 25 mM) and phenylalanine (2,4-D 1 mg/L + Ph 100 ppm) concentrations. **Conclusion:** The findings indicate that amino acid supplementation in tissue culture enhances the enzymatic antioxidant defense system in *Moringa oleifera* callus. This suggests a potential strategy for optimizing the production of bioactive compounds with antioxidant properties, which may have applications in pharmacology, laboratory medicine, and therapeutic research. Further studies are recommended to explore the biochemical pathways involved and the potential use of *Moringa oleifera* extracts in clinical settings

Keywords: Antioxidant Enzymes, 2,4- Dichlorophenoxyiacetic Acid, Benzyl Adanine, Tryptophan, Phenyl Alalanine, *Moringa Oleifera*, Callus.

INTRODUCTION

Moringa Oleifera L. is a fast-growing perennial and flowering plant. These plants belong to the family of (moringaceae) and include about 13 species. Their height ranges between (6-15 m) and their diameter ranges between (20-40 cm). Their wood is distinctive, relatively soft, from soft to Rough texture [1]. This tree can grow 6 months after planting, and its fruits are brown in color and hanging in the form of a capsule, about (20-45 cm) in size [2]. This tree has multiple benefits as it can be used as animal feed or cleaning agents (from leaf powder) [3]. It also has an important role in antioxidants and inhibitory activity against many types of bacteria and fungi [4]. The *Moringa oleifera* tree is found in the sub-Himalayan regions of northern West India and South America. *Moringa* is also widely known and used by humans for its health. It has earned its name as the "Miracle Tree" for its amazing healing powers For various diseases and even some chronic diseases [5] The Tissue culture is an alternative method for producing secondary metabolic compounds, such as alkaloids, flavonoids, and glycosides, including Gallic acid, Quercetin, Rutin and Kaempferol. Some cellular organelles and calluses resulting from cell culture or some parts of plants contain more active substances than those found in whole plants [6]. By controlling tissue culture, we can quantitatively control the amount of substances present in the cultured tissue, and control the toxicity associated with some of the resulting metabolic byproducts, especially in essential oils [7]. Oxidative stress results from exposure of the plant or cell to high concentrations of salinity for a long period, where the production of Reactive Oxygen Species (ROS), such as the Superoxide (O_2^-),

hydrogen peroxide (H_2O_2), and hydroxyl radicals (OH), resulting from incomplete reduction [8]. Active oxygen radicals are highly toxic to cells, as they negatively affect the functions of cellular membranes, damaging them, and damaging DNA, proteins, and chlorophyll [9]. Plants have a defense system to reduce the toxicity of these forms. This mechanism includes two types of systems: the first: non-enzymatic systems such as the Carotenoids and Ascorbate, second: enzymatic systems such as Superoxide dismutase (SOD), peroxidase (POD) and Catalase (CAT) [10].

METHODS AND MATERIALS

Assay of Peroxidase Activity

The assay was carried out by the method of Addy and Goodman (17). The reaction mixture consisted of 3ml of buffered pyrogallol [0.05 M pyrogallol in 0.1 M phosphate buffer (pH 7.0)] and 0.5 ml of 1% H_2O_2 . To this added 0.1 ml enzyme extract and O.D. change was measured at 430 nm for every 30 seconds for 2 minutes. The peroxidase activity was calculated using an extinction coefficient of oxidized pyrogallol (4.5 M/cm). The enzyme activity can be calculated according to the following equation: Enzyme activity (Units/L) = $(\Delta Abs \times Total\ assay\ volume) / (\Delta t \times \epsilon \times l \times Enzyme\ sample\ volume)$. Where Δt is the time of incubation (min), ΔAbs is the change in absorbance, ϵ is the extinction coefficient of substrates in units of $M^{-1} cm^{-1}$, and l is the cuvette diameter (1cm). Enzyme

activity (Unit) was defined as the amount of enzyme that oxidized 1 μ mol of substrate/min [11].

Catalase Activity Assay

0.2 ml serum was incubated in 1 ml of substrate (65 μ mol per ml H_2O_2 in 60 mmol/L sodium-potassium phosphate buffer, pH 7.4) at 37 °C for 1 min. The enzyme activity was suspended by adding 1 ml of 32.4 mM ammonium molybdate. The yellow absorption value of the molybdate complex and hydrogen peroxide was measured at 405 nm using a spectrophotometer. Catalase enzyme activity was calculated according to the following equation: C.A (KU/l) = (S-B1/B2-B3) * 271, C.A: Catalase activity (KU/l), S: Sample reading. B1: Blank 1 reading contained 1.0 ml substrate, 1.0 mL molybdate and 0.2 mL sample. B2: Blank 2 reading contained 1.0 ml substrate, 1.0 ml molybdate, and 0.2 ml of 60 mmol/L sodium-potassium phosphate buffer, pH 7.4. B3: Blank 3 reading contained 1.0 ml of 60 mmol/L sodium-potassium phosphate buffer pH 7.4, 1.0 mL molybdate and 0.2 ml of 60 mmol/L sodium-potassium phosphate buffer pH 7.4 [11].

Determination of Superoxide Dismutase Enzyme Activity

Superoxide dismutase activity was measured using colorimetric method by UV-VIS spectrophotometer. Principle: The principle of this method is based on the production of pyrogallol-quinone through a reactive intermediate, the semiquinone radical, and the ability of SOD to inhibit this reaction by radical dismutation. Pyrogallol-quinone is brown and absorbs visible light at 420 nm.

Reagents preparation: Tris buffer (pH 8.0): was prepared by dissolving 0.258 gm of tris and 0.111 gm of Ethylenediaminetetraacetic acid (EDTA) in dH_2O and completing the volume to 100 ml. Pyragallol solution (0.2 mM): was prepared by dissolving 0.0252 gm of pyragallol with 10 ml of HCl and completing the volume to 100 ml with dH_2O .

Assay Procedure

According to Marklund and Marklund [11], reaction mixture is consisting of 50 μ l of extract with 2 ml of tris buffer and 0.5 ml of pyragallol (0.2 mM) which absorbs light at 420 nm. Control solution contains the same materials except for the enzyme extract that was replaced by dH_2O . As a blank, dH_2O was used. Single unit of enzyme is defined as the amount of enzyme that is capable of inhibiting 50% of pyragallol oxidation. SOD activity was calculated using the following equation: SOD activity (u/ml) = $(V_p - V_s) / (V_p * 0.5) * (V_t / V_s) * n$, V_p = Auto oxidation rate of pyragallol (control), V_s = Auto oxidation rate of sample (with enzyme), V_t = Total reaction volume (ml), V_s =

volume of enzyme used for the assay (ml), n = dilution fold of the SOD sample, 0.5 = factor for 50% inhibition [12].

Results and Discussion

Table (1) We note that the activity of the Catalase enzyme increased in the callus of the *Moringa Oleifera* L. plant when the culture medium was treated hormones 2,4-D 1 + BA 0.5 and tryptophan [10], where the activity of the enzyme reached (122.84 U/gm.F.W), higher than the activity of the enzyme in callus grown on the culture medium treated with (2,4-D 1), (2,4 D 2), (2,4-D 2 +BA 2), While we note that the activity of the catalase enzyme also increased in the callus of the *Moringa Oleifera* L. plant when the culture medium was treated with the hormones 2,4.D1 + BA 0.5 and phenylalanine (50), where the enzyme activity reached (87.48 U/gm.F.W), higher than the enzymatic activity. In callus grown on culture medium treated with (2,4-D1), (2,4D2), (2,4-D2 + BA 2). This is consistent with *Sharma and Ramawat* [13]. Table (3) correct We note that the activity of the Superoxide dismutase (SOD) enzyme increased in the callus of the *Moringa Oleifera* L. plant when the culture medium was treated hormones 2,4.D 1 + BA 0.5 and tryptophan(10), where the activity of the enzyme reached (61.58 U/gm.F.W), higher than the activity of the enzyme in callus grown on the culture medium treated with (2,4-D 1), (2,4 D 2), (2,4-D 2 +BA 2), While we note that the activity of the SOD enzyme also increased in the callus of the *Moringa Oleifera* L. plant when the culture medium was treated with the hormones 2,4.D1 + BA 0.5 and phenyl alanine (50), where the enzyme activity reached (62.42 U/gm.F.W), higher than the enzymatic activity. In callus grown on culture medium treated with (2,4-D1), (2,4D2), (2,4-D2 + BA 2). This is consistent with Gupta.s. et al. [14]. Table (4) We note that the activity of the Peroxidase enzyme increased in the callus of the *Moringa Oleifera* L. plant when the culture medium was treated hormones 2,4.D 2 and tryptophan(25), where the activity of the enzyme reached (2.26 U/gm.F.W), higher than the activity of the enzyme in callus grown on the culture medium treated with (2,4-D 1), (2,4 D 1 + BA 0.5), (2,4-D 2 +BA 2), While we note that the activity of the peroxidase enzyme also increased in the callus of the *Moringa Oleifera* L. plant when the culture medium was treated with the hormones 2,4.D2 and phenyl alanine (100), where the enzyme activity reached (1.87 U/gm.F.W), higher than the enzymatic activity [15]. In callus grown on culture medium treated with (2,4-D1), (2,4D1 + BA 0.5), (2,4-D2 + BA 2). This is consistent with A. Bobrvkikh et al., [16].

Table 1: Effect of Different Treatments of Amino Acids on Catalase Activity (U/ gm.F.W).

Concentration (mg/L)	Tryptophan (U/ mg)			Phenyl Alanine (U/ mg)		
	5(mM)	10(mM)	25(mM)	50(ppm)	100(ppm)	200(ppm)
2,4-D (1)	81.07	113.74	59.46	51.45	63.04	55.79
2,4-D (2)	74.27	45.19	46.43	66.00	68.91	75.95
2,4-D (1) +BA (0.5)	61.03	122.84	51.90	87.48	70.26	50.79
2,4-D (2) +BA (2)	97.37	31.65	64.85	67.62	66.80	65.23
LSD (0.05)	8.126					

Table 2: The Effect of Different Treatments of Amino Acids on the Activity of the Superoxide Dismutase Enzyme (U/ gm.F.W) .

Concentration (mg/L)	Tryptophan (U/ mg)			Phenyl Alanine (U/ mg)		
	5(mM)	10(mM)	25(mM)	50(ppm)	100(ppm)	200(ppm)
2,4-D (1)	58.64	56.95	60.32	55.90	61.16	48.96
2,4-D (2)	50.43	52.33	58.64	58.85	55.06	57.79
2,4-D (1) +BA (0.5)	60.53	61.58	56.32	62.42	59.27	55.48
2,4-D (2) +BA (2)	60.74	60.32	58.64	61.79	58.21	59.06
LSD (0.05)	5.227					

Table 3: Effect of Different Treatments of Amino Acids on Peroxidase Activity (U/ gm.F.W).

Concentration (mg/L)	Tryptophan (U / mg)			Phenyl Alanine (U / mg)		
	5(mM)	10(mM)	25(mM)	50(ppm)	100(ppm)	200(ppm)
2,4-D (1)	1.94	2.19	1.68	0.89	1.35	1.56
2,4-D (2)	0.68	0.56	2.26	1.03	1.87	1.52
2,4-D (1) + BA (0.5)	1.19	1.63	0.58	0.96	0.75	0.68
2,4-D (2) +BA (2)	0.93	0.70	0.65	1.40	1.59	1.96
LSD (0.05)				0.341		

Conclusion

This research aims to know the different concentrations for the production of secondary compounds in the callus of the *Moringa Oleifera L.* plant and to know which concentration of amino acids gives the highest stress value by knowing the rate of activity of antioxidant enzymes in the callus of the *Moringa Oleifera L.* Through this study,

it became clear that the best concentration for producing the Catalase and SOD enzyme is 2,4-D 1 + BA 0.5 with tryptophan at a concentration of (10 mM) and also the same concentration with phenylalanine at a concentration of (50 ppm), As for the peroxidase enzyme the best concentration for its production is 2,4-D 2 with tryptophan at a concentration of (25 mM) and also with phenylalanine at a concentration of (100 ppm).

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