Response of Growth and Glucosinolate Derivatives to Spraying Tryptophan and Methionine on Arugula Leaves

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Abstract

The experiment was done during the agricultural winter season 2024 to measure the response of Arugula growth parameter, total glucosinolate, and their derivatives to spraying two amino acids Tryptophan and Methionine, and their combined treats. The experimental design was a randomized complete block design (R.C.B.D) with three replicates, and means were contrasted with the least significant difference test (L.S.D) at probability level 0.05.

The growth parameters refer to the significant superiority of spraying Methionine100 + Tryptophane 100 mg L^{-1} on leaves No., leaves area, total chlorophyll pigments, fresh and dry weight, fresh leaves yield, and reached the percentage increased in total glucosinolate, glucoerucin, glucoraphanin, progoitrin, and epiprogoitrin in 21.14, 27.75, 22.80, 22.65, and 22.75% respectively, while, treatment by spraying Tryptophane 100 mg L^{-1} was suggested to be significantly superior in leaves content of glucobrassicin, and 4-methyloxyglucobrassicin reached 5.88 and 3.70 mg g-1 DW respectively.

keywords: Tryptophan, Methionine, glucosinolate, Arugula, *Eruca vesicaria*.

Introduction

Arugula is a leafy vegetable that belongs to the Brassicaceae family, characterized by the abundance of glucosinolate compounds that give leaves a distinctive flavor and taste, and protective effects against several diseases, the most important are anticancer [1], antioxidant, antimicrobial, antiviral (including COVID-19), anti-inflammatory, anti-obesity, antidiabetic, and cardioprotective activity **[2]**. Glucosinolates were recently classified as a group of compounds that include many derivatives, as the structural description of about 90 compounds, and 49 of them have not yet been precisely identified **[3]**.

The research of plant nutrition and fertilization experiments has led to the possibility of controlling to stimulate some genes that enhance the production of some active compounds, including glucosinolate derivatives, whose concentrations in Brassicaceae plants are greatly affected by the abundance of sulfur and nitrogen **[4]**. It was noted that amino acids can contribute to this metabolic engineering of active compounds **[5]**, the amino acids that affected to produce glucosinolate derivatives are methionine, tryptophan, phenylalanine, and tyrosine **[6,7]** was found that the aliphatic glucosinolate derivatives increased by methionine, while the indole glucosinolates were increased by the influence of tryptophan and phenylalanine, whereas phenolic glucosinolate responded to add tyrosine.

Material and Methods

The experiment was conducted during the agricultural season of 2023/2024 on one private vegetable farm in Babylon Governorate. Table (1) shows the chemical and physical properties of the soil.

Table (1). Chemical and physical properties of soil

pH	O. M (%)	Ec (dsm^{-1})	Available Nutrients ($mgkg^{-1}$)			Silt (%)	Clay (%)	Sand (%)	Soil Texture
			N	P	K				
7.75	1.43	2.9	21.8	8.39	226.1	54.7	31.4	13.9	Silty-Clay

The field was prepared for cultivation by plowing, smoothing, and leveling, and during plowing, added fertilizers decomposed organic, triple superphosphate (52-44% P_2O_5), and potassium sulfate (53% K_2O) at a rate of 40 m^3 ha^{-1}, 100, and 80 kg ha^{-1} respectively **[8]**, the field was divided into three blocks, and then each block was divided into 9 experimental units with dimensions of 2 + 2 m. Arugula seeds (local variety) were tested for germination rate, which was 82%. The seeds were planted on November 2, 2023 in rows within the experimental units with a distance of 30 cm between rows and 10 cm between holes. All recommended service operations were carried out **[4]**. the experimental treatments were distributed according to a complete randomized block design (CRBD), and then means were compared according to the least significant difference test (LSD) at the probability level of 0.05 after data collection and statically analyzing **[9]**.

The experiment included foliage applications with two amino acids methionine, and tryptophan at concentrations 50 and 100 mg L^{-1} for each **[10]**. Three sprays of amino acids and their combinations were carried out after 30, 45, and 60 days of planting whereas the control treatment was sprayed with distilled water only **[11,12]**. The measure of some growth parameters was carried out after 80 days of planting, it is included leaf number, leaf area, total chlorophyll, and plant fresh and dry weight, after that, the plants were mowed to measure fresh leaf yield and evaluate the content of glucosinolate compounds and their derivatives.

The estimation of glucosinolate and their derivatives was carried out according to **[12]** in three stages, which included removing fat from the samples, then extracting the total glucosinolates using a separation column, after which the concentrations of glucosinolate compounds were estimated using an HPLC device.

Defatting was done as homogeneous samples were taken for each treatment, washed, dried, and ground, 1 g was taken, 10 ml of hexane was added to it, and left for 10 minutes, then filtered to remove the fat from it. The filtrate was excluded, the precipitate was taken, and 15 ml of 70% methanol alcohol was added to it. The samples were placed in a water bath for 5 minutes at a temperature of 70°C, then the samples were separated by a centrifugation device at a speed of 13,000 rpm^{-1} for 10 minutes, and the filtrate was taken, then the addition of alcohol to the precipitate, the water bath, and the centrifugation were repeated three times, and the filtrate was collected. Defatting was done as homogeneous samples were taken for each treatment, washed, dried, and ground, 1 g was taken, 10 ml of hexane was added to it, and left for 10 minutes, then filtered to remove the fat from it. The filtrate was excluded, the precipitate was taken, and 15 ml of 70% methanol alcohol was added to it. The samples were placed in a water bath for 5 minutes at a temperature of 70°C, then the samples were separated by a centrifugation device at a speed of 13,000 rpm-1 for 10 minutes, and the filtrate was taken, then the addition of alcohol to the precipitate, the water bath, and the centrifugation were repeated three times, and the filtrate was collected.

Total glucosinolate separation was done by filtration of 1 ml of sample then adding 0.75 ml of Aryl Sulfatase enzyme solution (prepared by 23 mg enzyme per 1 ml distilled water). A DEAE-Sephndex A25 column containing spherical particles coated with paraffin wax was used. After 16 hours, 2 ml of distilled water was added to wash the column and collect the filtrate containing glucosinolate compounds (wash it 4 times) and collect the filtrate. After that, 1 ml of the filtrate was taken and 1 ml of methanol was added, and stored in the refrigerator at 4°C.

The important glucosinolate derivatives in arugula leaves (Table 2) consist of Glucoerucin, Glucoraphanin, Progoitrin, Epiprogoitrin, Glucobrassicin, and 4methoxyglucobrassicin, these compounds have estimated the concentrations by using the high-performance liquid chromatography (HPLC) device. The stationary phase was an ODS-2 separation column with dimensions of 4.6 mm + 250 mm. The mobile phase included a mixture of ionic water and Acetonitrile solvent at a concentration of 20% (volume: volume) at a flow rate of 1 ml/min and a temperature of 35 °C. Colorimetric detection was carried out at the wavelength range of 190-370 nm. The retention time (RT) of glucosinolate derivatives appears in.

Table (2). Structures of glucosinolate derivatives isolated from Arugula leaves

PN	RT	MF	SR	TN	MW
1	2.19	$C_{11}H_{19}NO_{10}S_2$	2-Hydroxy-3-butenyl	Progoitrin	389.4
2	3.29	$C_{12}H_{23}NO_{10}S_3$	4-(Methylsulphinyl)butyl	Glucoraphanin	437.5
3	4.62	$C_{11}H_{19}NO_{10}S_2$	2(S)-Hydroxy-3-butenyl	Epiprogoitrin	389.4
4	6.87	$C_{17}H_{22}N_2O_{10}S_2$	4-methoxyindol-3-ylmethyl	4-methoxyglucobrassicin	478.5
5	7.94	$C_{12}H_{23}NO_9S_3$	4-(Methylthio)butyl	Glucoerucin	421.5
6	9.11	$C_{16}H_{20}N_2O_9S_2$	Indol-3-ylmethyl	Glucobrassicin	448.5

PN: peak number; RT: retention time; MF: molecular formula; SR: structure of R-groups; TN: trivial name; MW; molecular weight.

the optical absorption of the samples was calibrated according to the standard compounds of glucosinolate compounds (mg Kg^{-1}), the following equation was applied to calculate the concentrations of glucosinolate compounds as follows :

$$\text{Compound Conc. (mg Kg}^{-1}\text{)} = \frac{\text{Standard Compound Conc. (mg Kg}^{-1}\text{)} + \text{Sample Area} + \text{Dilution Factor}}{\text{Standard Compound Area} + \text{Sample Weight (g)}}$$

Results and Discussion

Growth Parameters

The result of the statistical analysis in Table (1) shows that the experimental treatments significantly affected in growth parameters of the arugula plant. At the level of single spraying with each of two amino acids, it was found that there was a significant superiority of spraying with methionine acid at a concentration of 100 mg L^{-1} in the traits of plant height, leave area, total chlorophyll content of leaves, fresh and dry weights of the plant, and fresh leaf yield amounted to 9.02 cm, 769.4 cm^2, 55.66 SPAD, 79.28 and 8.102 g $plant^{-1}$ and 2.642 kg m^2, respectively, compared to spraying with methionine acid at a concentration of 50 mg L^{-1} and tryptophan acid at concentrations of 50 and 100 mg L^{-1}. The lowest averages of the above traits were in the control treatment (spraying distilled water only), which amounted to 8.33 cm, 718.4 cm^2, 47.62 SPAD, 59.87 and 6.354 g $plant^{-1}$ and 1.995 kg m^2, respectively. This may be due to the role of methionine acid in the central dogma of molecular biology, through the translation stage of mRNA to all protein **[13]** , in addition increasing the production of growth hormones like the cytokinins and ethylene which works to increase root growth **[14]**, attract compounds produced through photosynthesis to the leaves that were exposed to spraying, also the methionine content of sulfur and nitrogen enhances the production of porphyrins **[15]** necessary to produce more chlorophyll pigments, that increased the dry matter content and leave yield.

Table (1): Response of Growth parameters of Arugula to Spraying Tryptophan and Methionine

Treatment	Leaves No. (Plant)	Leave Area (cm^2)	Total Chlorophyll (SPAD)	Fresh Weight (g plant)	Dry Weight (g plant)	Fresh Leaves Yield (Kg m^2)
Control	8.33	718.4	47.62	59.87	6.354	1.995
Met_{50}	9.52	819.8	50.43	71.62	7.415	2.387
Met_{100}	10.37	887.5	55.66	79.28	8.102	2.642
Try_{50}	8.64	751.3	48.27	66.90	6.899	2.230
Try_{100}	9.02	769.4	49.71	68.56	7.095	2.285
Met_{50} + Try_{50}	9.31	809.4	51.36	72.81	7.426	2.427
Met_{50} + Try_{100}	9.68	836.1	53.35	75.48	7.602	2.516
Met_{100} + Try_{50}	10.48	902.5	56.19	80.64	8.374	2.688
Met_{100} + Try_{100}	10.71	919.8	57.91	82.91	8.501	2.763
LSD (*P=0.05*)	0.09	7.73	0.55	0.73	0.06	6.95

It is also noted from Table (1) that spraying arugula plants with the two acids together at a concentration of 100 mg L^{-1} for each produced the highest means in growth traits, reaching 10.71 cm, 919.8 cm^2, 57.91 SPAD, 82.91 and 8.501 g $plant^{-1}$ and 2.763 kg m^2, respectively, compared to all treatments of combined spraying between the two acids and spraying each of them individually. This act may be attributed to the synergy between the effects of the two acids in increasing the means of the traits. Tryptophan is the starting material in the production of the auxin growth hormone indole-3-acetic acid **[16]**, which works to increase the longitudinal growth of the plant and enhance the activity of the meristem tissues of the leaves, thus increasing its area, which allows an increase in the light intercepted by the leaves and the production of an abundance of dry matter and leave yield.

Total Glucosinolate and Their Derivatives

Table (2) shows that the glucosinolates and their derivatives were significantly affected by experimental treatments in arugula leaves. At the level of single spraying with each of two amino acids, it was found that there was a significant superiority of spraying with methionine at a concentration of 100 mg L^{-1} in the compounds glucoerucin, glucoraphanin, progoitrin, epiprogoitrin, and total glucosinolate amounted to 11.90, 22.41, 9.02, 7.44, and 48.33 mg Kg^{-1} D.W, respectively, while, spraying with tryptophane at a concentration of 100 mg L^{-1} was recorded highest means in the compounds glucobrassicin and 4-methoxy glucobrassicin amounted to 5.88 and 3.70 mg Kg^{-1} D.W, respectively, compared to spraying with methionine and/or tryptophan in all treatments, whereas the control treatment harvested the lowest means of the compounds glucoerucin, glucoraphanin, progoitrin, epiprogoitrin, glucobrassicin, 4-methoxy glucobrassicin, and total glucosinolate, which amounted to 9.06, 18.59, 7.41, 5.57, 4.30, 2.29 and 40.56 Kg^{-1} D.W, respectively.

Table (2): Response of Total Glucosinolate and Their Derivatives (mg g^{-1} D.W) of Arugula to Spraying Tryptophan and Methionine

Treatment	Glucoerucin	Glucoraphanin	Progoitrin	Epiprogoitrin	Glucobrassicin	4methoxyglucobrassicin	Total Glucosinolate
Control	9.06	18.59	7.41	5.57	4.30	2.29	40.56
Met_{50}	10.78	21.90	8.85	6.70	4.44	2.45	46.85
Met_{100}	11.90	22.41	9.02	7.44	4.55	2.58	48.33
Try_{50}	9.95	20.29	8.15	6.15	5.58	3.52	45.69
Try_{100}	10.26	20.91	8.42	6.36	5.88	3.70	47.91
Met_{50} + Try_{50}	10.80	21.94	8.87	6.72	5.22	3.08	48.25
Met_{50} + Try_{100}	11.09	22.49	9.11	6.90	5.39	3.30	49.87
Met_{100} + Try_{50}	12.34	23.15	9.34	7.02	4.60	2.54	49.28
Met_{100} + Try_{100}	12.54	24.08	9.58	7.21	4.99	2.86	51.43
LSD (*P=0.05*)	0.10	0.19	0.08	0.06	0.05	0.03	0.52

The results in Table (2) also indicate that spraying arugula plants with both amino acids together at concentrations of 1

00 mg L^{-1} for each one led to a significant superiority in the leaf content of compounds glucoerucin, glucoraphanin, progoitrin, epiprogoitrin, and total glucosinolate amounted to 12.54, 24.08, 9.58, 7.21, and 51.43 mg Kg^{-1} D.W, respectively, compared to control treatment that treated with distilled water only, which recorded the lowest values in total glucosinolate and their derivatives. These results indicate the important role of amino acids in the biosynthesis of building various biological compounds. It is noted that the methionine participates in the metabolic pathways for the production of aliphatic glucosinolates in three stages: initiation structure, elongation, and modification of side chain of glucosinolate and their derivatives **[17]**, which include glucoerucin, glucoraphanin, progoitrin, epiprogoitrin, and total glucosinolates. It is also the amino acid tryptophan that led to an increase in the concentration of indole glucosinolate derivatives glucobrassicin and 4-methoxy glucobrassicin, indicating that this acid participated as a raw material in preparing the biosynthesis of these compounds with the indole ring through several oxidation-reduction reactions and isomerization.

Conclusion

Arugula plant responds to foliar fertilization with amino acids by increasing growth indicators and leaf yield and expanding the leaf content of indolic or/and aliphatic glucosinolates, depending on the type and concentration of the amino acid. This response may give us the hope to design the components of arugula leaves or other medicinal plants according to the treatment requirements.

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