Response of the content of Antioxidants of Swiss chard to treatment with potassium and phenylalanine

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Abstract

The experiment was conducted at the research station of the Faculty of Agriculture / University of Kufa / Najaf Governorate during the autumn agricultural season of 2023 to demonstrate the effect of spraying with potassium in three concentrations (0, 2 and 4) g L-1and Phenylalanine also in three concentrations (0, 50 and 100) mg L-1 on the qualitative content indicators of chard plants. A factorial experiment was designed in Randomized Complete Block Design (RCBD), the experiment included 27 experimental units, where the land allocated for the experiment was divided into three sectors, and each sector contains 9 treatments (experimental unit) and the number of plants in the experimental unit is 36 plants, the averages of the treatments were compared according to the Least Significant Difference (LSD) test and under the probability level of 0.05. The results showed that the concentration of 2 g L-1 was superior to the leaf content of total chlorophyll, and the concentration of 4 g L-1 was superior to the leaf content of anthocyanin, quercetin, rutin and gallic acid compared to the other two concentrations, with the same concentration being superior to the leaf content of Kaempferol and Ferulic acid compared to the comparison treatment. Phenylalanine concentrations had a significant effect on the leaf content of antioxidants. The interaction coefficients had a significant effect on the studied indicators.

Keywords: Beta vulgaris, Potassium, Phenylalanine, Antioxidants content

Introduction

Leafy vegetables contain abundant amounts of antioxidant compounds, which are important in maintaining human health and preventing diseases ([8], [19], including Swiss chard Beta vulgaris Subsp. cicla L., which belongs to the Chenopodiaceae family, which is consumed at a high rate by the Iraqi family, and contains large concentrations of compounds with high activity in combating free radicals (Free-radical Scavenging) and antioxidants, such as phenolic acids (Caffeic, p-Cumaric, and Syringic) and flavonoids (glycosides derived from apigenin, kaempferol, and quercetin from apigenin) ([13]; [15]; [9]. Increasing the antioxidant content requires providing ideal plant growth, including plant nutrition. Potassium is one of the essential elements that affect most of the chemical, biological and physiological processes in the plant ([20], in addition to its significant contribution to the photosynthesis process, enzyme activation, and maintaining cell swelling and ionic balance ([11]. [10] indicated that potassium deficiency will reduce the flavonoid content in many medicinal plants due to limited or low enzyme activities in the flavonoid synthesis process. Foliar spraying with the main initiator of active compounds can increase their concentration within plant tissues, including the amino acid Phenylalanine. [1] explained that in higher plants, the amino acid Phenylalanine is a substrate for both primary and secondary metabolic pathways and is also an initiator for many phenolic compounds such as flavonoids, lignin, condensed tannins,

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and benzenoid phenylpropanoid volatiles. [6] also indicated that Phenylalanine is an initiator of secondary products in the plant, and Phenylalanine is a bioactivator, and is the basis for the metabolism of many active compounds such as phenols, flavonoids, and terpenes, which makes it play an important role in enhancing auxins and gibberellins, as well as its role as a source of nitrogen necessary for building proteins and enzymes that enhance plant growth and productivity ([3.[

The research aims to clarify the role of the amino acid Phenylalanine in the antioxidant content of the plant as an important initiator. In addition to demonstrate the role of potassium in regulating photosynthesis enzymes and thus increasing the antioxidant content.

Materials and Methods

The planting season and date must be mentioned precisely

The field experiment for the cultivation of Swiss chard was carried out during the 2023 autumnal agricultural season at the research station belong to the Faculty of Agriculture / University of Kufa in Najaf Governorate, where soil preparation operations were carried out in full, including ploughing and smoothing, and field soil samples, the physical and chemical properties of which are shown in Table 1, were analysed in the laboratories of postgraduate studies / College of Agriculture / University of Kufa. The experiment included 27 experimental units, where the land allocated for the experiment was divided into three sectors, and each sector contains 9 treatments (experimental unit), and cultivation was carried out in panels where each panel represents an experimental unit, and the area of each panel is (1.5 m x 1.5 m), and thus the area of the experimental unit is 2.25 m2, and 36 plants were planted in the experimental unit (for each treatment), the distance between one plant and another is 0.25 m, and thus the number of plants for the experiment becomes 972 plants, noting that cultivation within each panel was carried out in lines, as the distance between one line and another is 0.25 m and the distance between one panel and another is 0.75 m. The seeds were planted directly in the field on 28/10/2023, and the seeds were germinated on 11/4/2023. The individualization and patching processes were carried out in the second week after germination from planting, with manual and periodic weeding of all treatments during the plant growth season. A drip irrigation system was used to irrigate the experiment, and fungicides and insecticides were sprayed preventively to avoid fungal and insect infections expected to occur directly with planting. A factorial experiment was designed in Randomized Complete Block Design (RCBD) and the averages of the treatments were compared according to the Least Significant Difference (LSD) test at a probability level of 0.05 with three replicates and two factors :

Factor 1: The first factor included spraying with three concentrations of potassium in the form of NPK fertilizer (0-50-0) and with three concentrations (0, 2 and 4) g L-1. The spraying process was repeated three times. The first spray was after 15 days of planting in the field, the second after 10 days of the first spray, and the third after 20 days of the first spray. The second factor included spraying with the amino acid (Phenylalanine) at three concentrations (0, 50 and 100) mg/L-1, at a rate of three sprays. The first spray was 15 days after planting in the field, the second 10 days after the first spray, and the third 20 days

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after the first spray. A number of antioxidants were estimated, namely the total chlorophyll content of the leaves according to the method of [7] and the anthocyanin pigment as stated in the method of [16], Quercetin, Rutin, Kaempferol, Gallic acid and Ferulic acid, and the active compounds were estimated according to the method followed by [12], which is summarized as follows:

Sample extraction:

3g of finely crushed samples were mixed with 60 ml of methanol/water (40/60) for 24 hours, the mixture was filtered, and the filtrate was concentrated under reduced pressure (40 °C) to a volume of 5 ml, then this solution was hydrolysed using 5 ml of NaOH (2 normal) for 30 minutes. The pH of the mixture was adjusted to pH 7.00 using HCl (2 normal) and the phenolic acids were extracted by liquid/liquid extraction using ethyl acetate (20 mL × 3), then the extracts were collected, and the ethyl acetate was removed under reduced pressure, then the residue was dissolved in 7 mL of methanol and 10 μL was taken and analysed by HPLC.

HPLC Separation Conditions:

An antioxidants are measured in Ministry of Science and Technology laboratories. High performance liquid chromatography (HPLC) was performed on a SYKAM HPLC system (Germany) equipped with a C18-ODS column (250 mmol × 4.6 mmol, 5 µmol), samples (100 µl) were injected into the system. The mobile phase consisted of 95% acetonitrile + 0.01% trifluoroacetic acid (80:20 v/v) at 1 ml/min, and the detection of phenolic compounds was performed using a UV-visible detector at 278 nm .

Results and discussion:

The results of Table 1 showed that potassium had a significant effect on the total chlorophyll content of the leaves, as the concentrations (0 and 2) g L-1 of potassium significantly outperformed the concentration of 4 g L-1 and gave the highest rate of (63.514 and 63.338) mg 100 g-1 fresh weight, for the two concentrations respectively, with no significant differences between them. It was also found that the concentration of 4 g L-1 significantly outperformed the other two concentrations in the leaf content of anthocyanin, Quercetin and Rutin, with rates of (8.503 mg 100 g-1 fresh weight, 27.15 mg kg-1 dry weight and 32.09 mg kg-1 dry weight) for the above traits respectively.

As for the Phenylalanine spray, the data in Table 1 showed that the concentration of 50 mg L-1 was significantly superior to the other two concentrations (0 and 100) mg L-1 in the total chlorophyll content of the leaves and gave the highest rate of 65.435 mg 100 g-1 fresh weight. As for the anthocyanin content of the leaves, the concentrations (0 and 100) mg L-1 were significantly superior to the concentration of 50 mg L-1 and gave the highest rate of (8.175 and 8.333) mg 100 g-1 fresh weight, for the two concentrations respectively, while the concentration of 100 mg L-1 was significantly superior to the concentrations (0 and 50) mg L-1 and gave the highest rate of the Quercetin content of the leaves and reached 26.16 mg kg-1 dry weight, and the concentrations (100 The interaction treatment (4 g L-1 of potassium with a concentration of 0 mg L-1 of Phenylalanine) significantly outperformed all interaction treatments and gave the highest rate of chlorophyll content in leaves, reaching 68.653

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mg 100 g-1 fresh weight, while the interaction treatment (4 g L-1 of potassium with a concentration of 100 mg L-1 of Phenylalanine) significantly outperformed the leaf content of anthocyanin and Quercetin Rutin, with rates reaching (8.927 mg 100 g-1 fresh weight and 28.38 mg kg-1 dry weight). and 33.59 mg kg-1 dry weight), respectively.

The results of Table 2 showed that the two experimental factors had a significant effect on the leaf content of Kaempferol, Gallic acid, and Ferulic acid. Potassium at a concentration of 4 g/L-1 of potassium significantly outperformed the comparison treatment in the leaf content of Kaempferol and Ferulic acid and gave the highest rate, reaching (34.30 and 16.34) mg kg-1 dry weight, respectively. The concentration of 4 g/L-1 of potassium significantly outperformed the leaf content of Gallic acid over the other two concentrations and gave the highest rate, reaching 24.23 mg kg-1 dry weight. As for spraying with Phenylalanine, the concentration of 100 mg L-1 of Phenylalanine, significantly outperformed the comparison treatment in the leaf content of Kaempferol, Gallic acid and Ferulic acid and gave the lowest values of (33.13, 23.34 and 15.33) mg kg-1 dry weight, respectively. As for the interaction between the two factors, the interaction treatment (4 g L-1 of potassium with a concentration of 100 mg L-1 of Phenylalanine) significantly outperformed in the leaf content of Kaempferol, Gallic acid and Ferulic acid, compared to some interaction treatments. Discussion: The significant effect achieved by potassium, especially the concentration of 4 g L-1, on the qualitative content indicators is due to the fact that it is one of the important and required elements for plants, so adequate and appropriate nutrition with this element leads to positive quantitative and qualitative changes ([4], as well as determining the taste in many vegetables through the number of solids, especially sugars and organic acids, in addition to volatile compounds whose biological composition and concentrations are affected by the addition of potassium ([21], in addition to studies indicating that potassium deficiency leads to a decrease in the accumulation of phenolic acids ([2] and flavonoids ([5], which was confirmed by Liu et al. (2010) who showed that potassium deficiency will reduce the content of flavonoids in many medicinal plants due to limited or low enzyme activities in the process of flavonoid synthesis, and what [22] confirmed that there are many studies that indicated that potassium fertilizers can affect On the content of total phenolics and flavonoids in plants, [18] found that spraying pepper plants with a mixture of amino acids led to an increase in the efficiency of the photosynthesis process, thus giving better vegetative growth and increasing the number of fruits and the total yield, in addition to improving the qualitative characteristics of the fruits, such as increasing the percentage of sugars, total soluble solids, and the content of total phenolics, as well as the efficiency of antioxidants. As for the significant effect achieved by Phenylalanine concentrations on the antioxidant content indicators, it is due to the fact that in higher plants, the amino acid Phenylalanine is considered a substrate for both primary and secondary metabolic pathways, as well as an initiator for many phenolic compounds such as Flavonoids, lignin, condensed tannins, and ([1], in addition to being an essential amino acid for plant growth and development, and acts as an initiator for many molecules important in plant growth, reproduction, and defense against abiotic and biotic stresses, including phenylpropanoid, Flavonoids, Anthocyanins,

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lignin, tannins, and Salicylates ([14], which was confirmed by [17] when he studied the effect of foliar spraying with Phenylalanine on growth, yield, oil, and content of some substances. The effective value of dill plant for the seasons 2018-2019 and 2019-2020 was determined, as three concentrations of Phenylalanine (0, 75 and 150) mg L-1 were used, and it was found that the concentration of 150 mg L-1 was significantly superior to the other two concentrations in the content of total carbohydrates, nitrogen, phosphorus, potassium and protein, as well as the content of the green group of antioxidants, represented by the content of total chlorophyll, total phenols, total flavonoids, vitamin C and carotene for both seasons, noting that the concentration of 75 mg plant-1 was significantly superior to the comparison treatment (which gave the lowest rates) in the above-mentioned characteristics .

Table 1. Effect of foliar application of potassium and Phenylalanine on content of leaves of Total chlorophyll, Anthocyanin, Quercetin and Rutin

Total chlorophyll content of leaves (mg $100g^{-1}$ FW)				
Phenylalanine (mg l^{-1})	Potassium (gm l^{-1})			
	0	2	4	Phenylalanine average
0	61.704	54.757	68.653	61.704
50	64.953	67.374	63.976	65.435
100	63.886	67.884	55.454	62.408
Potassium average	63.514	63.338	62.694	
LSD (P≤0.05)	K= 0.629	Phen. = 0.629	Interaction= 1.291	
Anthocyanin content of leaves (mg $100g^{-1}$ FW)				
Phenylalanine (mg l^{-1})	Potassium (gm l^{-1})			
	0	2	4	Phenylalanine average
0	8.248	7.926	8.350	8.175
50	6.959	6.993	8.232	7.394
100	7.994	8.079	8.927	8.333
Potassium average	7.734	7.666	8.503	
LSD (P≤0.05)	K= 0.445	Phen. = 0.445	Interaction= 1.063	
Quercetin content of leaves (mg kg^{-1} DW)				
Phenylalanine (mg l^{-1})	Potassium (gm l^{-1})			
	0	2	4	Phenylalanine average
0	20.32	22.55	25.72	22.86
50	21.40	24.65	27.35	24.47
100	23.40	26.70	28.38	26.16
Potassium average	21.71	24.63	27.15	
LSD (P≤0.05)	K= 1.643	Phen. = 1.643	Interaction= 3.319	
Rutin content of leaves (mg kg^{-1} DW)				
Phenylalanine (mg l^{-1})	Potassium (gm l^{-1})			
	0	2	4	Phenylalanine average

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0	25.38	27.49	30.40	27.76
50	26.28	29.51	32.29	29.36
100	28.61	28.61	33.59	30.27
Potassium average	26.75	28.53	32.09	
LSD ($P \leq 0.05$)	K= 2.183	Phen. = 2.183	Interaction= 4.338	

Table 1. Effect of foliar application of potassium and Phenylalanine on content of leaves of Kaempferol, Gallic acid and Ferulic acid.

Kaempferol content of leaves (mg 100g^{-1} FW)

Phenylalanine (mg l^{-1})	Potassium (gm l^{-1}) 0	2	4	Phenylalanine average
0	27.57	29.77	32.77	30.04
50	28.55	31.33	34.74	31.54
100	30.44	33.56	35.39	33.13
Potassium average	28.85	31.55	34.30	
LSD ($P \leq 0.05$)	K= 2.986	Phen. = 2.986	Interaction= 5.021	

Gallic acid content of leaves (mg 100g^{-1} FW)

Phenylalanine (mg l^{-1})	Potassium (gm l^{-1}) 0	2	4	Phenylalanine average
0	17.17	19.59	22.35	19.70
50	18.60	21.72	24.72	21.68
100	20.72	23.70	25.61	23.34
Potassium average	18.83	21.67	24.23	
LSD ($P \leq 0.05$)	K= 2.426	Phen. = 2.426	Interaction= 3.656	

Ferulic acid content of leaves (mg kg^{-1} DW)

Phenylalanine (mg l^{-1})	Potassium (gm l^{-1}) 0	2	4	Phenylalanine average
0	9.58	11.36	14.53	11.82
50	10.32	13.56	16.65	13.51
100	12.75	15.39	17.85	15.33
Potassium average	10.88	13.44	16.34	
LSD ($P \leq 0.05$)	K= 3.009	Phen. = 3.009	Interaction= 5.514	

Conclusion :

Swiss chard is a leafy vegetable belonging to the cruciferous family which characterized by its distinctive content of nutritionally and medically important antioxidants, including flavonoids and others. Increasing the concentration of antioxidants requires providing appropriate nutrition, including potassium, which affects most of the chemical, biological and physiological processes in the plant, as well as the necessity of treating it with the main initiator of flavonoids, which is Phenylalanine, which is a starter for many phenolic compounds, including anthocyanin,

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Quercetin, Rutin, Kaempferol, Gallic acid and Ferulic acid
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