Effect of spraying with aminolevulinic acid and phosphorus on some vegetative characteristics of Spinacia oleracea L.

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Abstract

The experiment was conducted at the research station of the Department of Horticulture and Landscape Engineering at the College of Agriculture, University of Diyala, for the agricultural season 2023-2024 to study the effect of spraying with aminolevulinic acid at three concentrations of 0, 50, and 100 mg L-1 (M1, M2, M3) and phosphorus at three concentrations of 0, 1, and 2 ml L-1 (P1, P2, P3) on some vegetative traits of two cultivars of local and Egyptian spinach (V1 and V2). The treatment M3 was significantly superior in leaf chlorophyll content, while treatment M2 recorded the highest values in both plant height and total number of leaves. The treatment P3 was superior in plant height and total number of leaves, whereas the cultivar V2 was superior in both leaf chlorophyll content and plant height. The interaction treatment P2M3 gave the highest leaf chlorophyll content, while treatment P2M2 gave the highest values in plant height and total number of leaves. The interaction treatment M3V2 was superior in leaf chlorophyll content, while the treatment M2V1 was superior in plant height and total number of leaves. As for the interaction treatment P3V2, it was superior in all studied traits. The P2M3V1 treatment was superior in the chlorophyll content of the leaves, as it gave a concentration of 21.10 mg g-1, and the P2M2V1 treatment was superior in the plant height and the total number of leaves per plant, which reached 35.60 cm and 18.26 leaves per plant-1, respectively. The lowest values were for the treatment P1M1V1, which reached 15.78 mg g-1, 26.00 cm, and 12.83 leaves per plant-1, respectively for the above traits.

Keywords. Spinach, aminolevulinic acid and phosphorus

Introduction

Spinach (Spinacia oleraceae L.) is a high-nutritional vegetable crop belonging to the Chenopodaceae family. Its original habitat is the Asian region, mostly Iran. From here, its cultivation spread to China in 674 BC, and then its cultivation spread to the regions of Europe during the 12th century (10). The cultivated area in the world is estimated at about 937829 hectares, while in Iraq the cultivated area is 500 hectares, with a total production rate of about 3198 tons per year (13). Foliar spraying with nutrient solutions has great importance to agricultural crops, as it improves their root, vegetative, and chemical characteristics, especially the spraying of microelements. It has a direct and effective contribution to the formation of chlorophyll and protein, increasing the rates of cell elongation and division, and increasing the surface leaf area of plants (5). To improve the vegetative characteristics, a number of treatments were used, the first of which was foliar spraying with aminolevulinic acid (ALA), which represents the main precursor for the biosynthesis of all porphyrin compounds, including heme, phytochrome, chlorophyll, and vitamin B12, where it works as a new plant hormone in addition to its work as a good substance for regulating plant

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growth (PGR), while in recent years, this acid has received a lot of attention for use as a plant stimulant under different stress conditions and its effect on the antioxidant system, photosynthesis, and other uses and effects (20)(15). Phosphorus is one of the most important macronutrients that plants need to complete their life cycle; it plays many roles in plants, such as in the germination process due to its importance in producing the energy needed for that process, and the phosphorus is one of the elements that plants need in large quantities (6). Phosphorus in the soil is exposed to many reactions, as it is transformed from the form ready for absorption to the form not ready, and the efficiency of phosphate fertilizers does not exceed 25-30% due to its exposure to either adsorption or precipitation (17). The research aims to improve the vegetative characteristics of two cultivars of spinach using foliar spraying with different levels of 5-aminolevulinic acid and phosphorus and to identify the best cultivar.

Materials and methods

The field experiment was carried out at the research station of the Department of Horticulture and Landscape Engineering of the College of Agriculture, University of Diyala, for the agricultural season 2023-2024 to study the effect of spraying with 5-aminolevulinic acid and phosphorus on the organic and mineral content of two cultivars of spinach. The field was prepared by removing weeds and smoothing and leveling the soil, then dividing it into 54 plots with an area of 1 m × 1 m for one plot and with three replicates, each replicate representing 18 plots, and leaving half a meter between them as a walkway to facilitate service operations, and the irrigation was by drip, Random samples were taken from soil for analysis and sent to the Soil Division in the Agriculture Directorate in Diyala Governorate (Table 1).The seeds of both cultivars were planted directly in the field and in rows on 7/11/2023, with a distance of 20 cm between the lines and 10 cm between the plants, as three seeds were placed in each hole, and after the appearance of the first true leaf for plants, they were reduced to one plant, at a rate of 60 plants per experimental unit. 66(21). The statistical program SAS was used to analyze the data to study the effect of three factors, 5-aminolevulinic acid, phosphorus, and the two cultivars and their interactions according to a factorial experiment (3×3×2) and implemented in a randomized complete block design (RCBD) with three replicates. The averages were compared according to Duncan's multiple range test at a probability level of 0.05 (4.(

Study factors

aminolevulinic acid

This factor includes three concentrations (0, 50, and 100 mg/L) and is symbolized as M0, M1, and M2, respectively.

Phosphorus

This factor includes three concentrations (0, 1, and 2 mL/L) and is symbolized as P1, P2, and P3, respectively.

Two cultivars of spinach

This factor includes two cultivars, V1, which represents the local cultivar (Iraqi), and V2, which represents the Egyptian cultivar.

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Table 1. The physical and chemical properties of soil

Measurements	Value	Unit of measurement
Texture of soil	Loamy	-
Clay	20.8	g. kg^{-1}
Silt	18.56	g. kg^{-1}
	60.64	g. kg^{-1}
PH	7.1	
EC	1.3	$ds.m^{-1}$
N	94.5	mg. kg^{-1}
P	15	mg. kg^{-1}
K	94	mg. kg^{-1}
Organic matter	2.32	%
CaCo3	23.6	g. kg^{-1}

The studied characteristics

Leaf chlorophyll content (mg 100 g-1(

Samples were taken from fully grown leaves before the first harvesting. 500 mg of fresh weight of these leaves were taken and placed in dark colored containers, and 20 ml of 80% acetone was added to them. The containers were left in a dark place for two days, and the shaking process was repeated until the chlorophyll was completely extracted. As the final volume of the extraction solution reached 50 ml g-1, the chlorophyll content was estimated according to the method (14), and the absorption of the filtrate for light at wavelengths 663,654 nm was read by a spectrophotometer, and the following equations were used to calculate the amount of chlorophyll:

Chlorophyll a = (9.78 × A663) - (0.99 × A645(

Chlorophyll b = (21.4 × A645) - (4.65 × A663(

Total Chlorophyll = Chlorophyll a + Chlorophyll b

Where A663 and A645 represent the readings of the device at wavelengths 663 and 645 nm, respectively.

Plant height (cm(

The height of five plants and for all treatments was measured using a tape measure from the beginning of the soil surface to the top of the plant. The measurement was made before the first harvesting on 29/12/2023.

Total number of leaves per plant (leaf plant-1(

The number of leaves was calculated for five plants and for all treatments, and their average was calculated. The measurement was made before the first harvesting on 29/12/2023.

Results and discussion

Leaf chlorophyll content (mg g-1(

The results of Table 2 showed a significant effect of spraying with 5-aminolevulinic acid on the total chlorophyll content of leaves, as treatment M3 was superior, reaching 19.91 mg g-1, while the control treatment gave the lowest content, reaching 16.82 mg g-1, which did not differ significantly from treatment M2. As this acid is an essential compound in the precursors that enter into biosynthesis, such as chlorophyll, phytochrome, and vitamin B12, it is necessary for it to participate in vital processes such as photosynthesis and respiration, and thus it works to increase the chlorophyll content of leaves (18) (19). The results of the same table indicated that there

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was no significant effect of different concentrations of phosphorus on this trait. The results also showed for the same trait that there was a significant effect of the varieties, as the V2 variety was superior in giving the highest total chlorophyll content of 18.54 mg g-1, while the V1 variety gave the lowest content of 17.69 mg g-1. The superiority of the Egyptian variety is due to the efficiency of the variety in the photosynthesis process and the difference in its genetic composition from the local variety. The binary interaction between aminolevulinic acid and phosphorus had a significant effect on this trait, as treatment P2M3 was superior and gave the highest total chlorophyll content of 20.19 mg g-1, while the control treatment gave the lowest content of 16.03 mg g-1. As for the interaction between aminolevulinic acid and the varieties, treatment M3V2 gave the highest chlorophyll content of 20.19 mg g-1, while treatment M1V1 gave the lowest content of 16.69 mg g-1. As for the binary interaction between phosphorus and the varieties, there was a significant effect, as treatment P3V2 gave the highest total chlorophyll content of 19.11 mg g-1, while treatment P1V1 gave the lowest content of 17.28 mg g-1. As for the triple interaction between 5-aminolevulinic acid, phosphorus, and varieties, the interaction treatment P2M3V1 outperformed with the highest total chlorophyll content of 21.10 mg g-1, while the treatment P1M1V1 gave the lowest content of 15.78 mg g-1.

Table 2. Effect of spraying with aminolevulinic acid and phosphorus and their interaction on the leaves content of chlorophyll for two spinach cultivars (mg g-1(

Cultivars	Phosphorus (ml L^{-1})	aminolevulinic acid (mg L^{-1})			Interaction between cultivars and phosphorus
		M1	M2	M3	
V1	P1	15.87 f	17.30 def	18.67 b-e	17.28 C
	P2	16.53 ef	16.37 ef	21.10 a	18.00 Abc
	P3	17.66 c-f	16.64 ef	19.10 a-d	17.80 Bc
V2	P1	16.19 f	17.15 def	20.55 ab	17.96 Abc
	P2	16.78 ef	19.64 abc	19.27 a-d	18.56 Ab
	P3	17.91 c-f	18.68 b-e	20.75 ab	19.11 A
Cultivars averages	V1	16.69 c	16.77 c	19.62 ab	17.69 B
	V2	16.96 c	18.49 b	20.19 a	18.54 A
Phosphorus averages	P1	16.03 c	17.23 bc	19.61 a	17.62 A
	P2	16.65 bc	18.00 b	20.19 a	18.28 A
	P3	17.78 b	17.66 b	19.92 a	18.45 A
aminolevulinic acid averages		16.82 B	17.63 B	19.91 A	

*M1 = 0 mg L-1, M2 = 50 mg L-1, M3 = 100 mg L-1 and P1 = 0 ml L-1, P2 = 1 ml L-1, P3 = 2 ml L-1 and V1 = local cultivar, V2 = Egyptian cultivar.

Plant height (cm(

The results of table 3 showed a significant effect on plant height when spraying with 5-aminolevulinic acid, as treatment M2 outperformed and gave the highest height of 32.76 cm, while treatment M1 recorded the lowest height of 30.05 cm. This acid improves the photosynthesis process, and by increasing it, food in the plant increases and thus growth

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increases. In addition to the emergence of this acid as a new plant hormone and working as a good substance to regulate growth in the plant as a result of its work in improving photosynthesis in addition to its work as a hormone, this leads to cell growth and elongation and thus an increase in plant height. These results are consistent with (7) in their study of the spinach crop, as treatment with amino acids led to a significant increase in the chlorophyll content of the leaves. The results also indicated a significant effect on this trait when spraying with different concentrations of phosphorus. Treatment P3 outperformed and gave the highest height of 34.14 cm, while the control treatment achieved the lowest plant height of 27.21 cm. Phosphorus is one of the major nutrients and is an essential component of nucleic acids, phospholipids, and the energy compound ATP, which is responsible for energy transfer, in addition to its entry into the composition of the enzyme cofactors NAD and NADP, on which many physiological processes of the plant depend, such as respiration, photosynthesis, acid and glycolysis construction. Phosphorus has an important role in carbohydrate metabolism and the formation of materials resulting from photosynthesis, thus improving it. Phosphorus is necessary to release energy, which is important for the vital processes of the plant. These important effects of phosphorus make it necessary to improve vegetative growth, whether for spinach or other crops. Therefore, it is necessary to provide the plant with sufficient amounts of this element(1) (8). These results are consistent with (3) in their study on the cabbage crop by increasing the chlorophyll content and the number of leaves. Also, the V2 cultivar was superior and recorded the highest height of 31.35 cm, while the V1 cultivar gave a lower plant height than the V2 cultivar, reaching 31.24 cm. These results are consistent with (3) in their study of the spinach crop. There was a significant effect of the binary interaction between aminolevulinic acid and phosphorus on the plant height trait, as the P2M2 treatment was superior with the highest height of 35.60 cm, and the comparison treatment gave the lowest plant height of 26.06 cm. As for the interaction between aminolevulinic acid and the cultivars, the interaction treatment M2V1 was superior with a height of 32.80 cm, while the interaction treatment M1V2 recorded the lowest plant height of 30.02 cm. The binary interaction between phosphorus and the cultivars had a significant effect on the same trait, as the interaction treatment P3V2 was superior with the highest height of 34.22 cm, while the lowest height was in the P1V1 treatment, which reached 27.17 cm. The triple interaction between aminolevulinic acid, phosphorus, and the cultivars affected the plant height, as the treatment P2M2V1 recorded the highest height of 35.60 cm, while the lowest plant height was in the treatment P1M1V1, which reached 26.00 cm.

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Table 3. Effect of spraying with aminolevulinic acid and phosphorus and their interaction on the plant height for two spinach cultivars (cm(

Cultivars	Phosphorus (ml L^{-1})	aminolevulinic acid (mg L^{-1})			Interaction between cultivars and phosphorus
		M1	M2	M3	
V1	P1	26.00 g	27.73 f	27.80 f	27.17 C
	P2	30.40 de	35.60 a	31.46 cd	32.48 B
	P3	33.66 b	35.06 a	33.46 b	34.06 A
V2	P1	26.13 g	27.43 f	28.20 f	27.25 C
	P2	30.20 e	35.60 a	31.93 c	32.57 B
	P3	33.93 b	35.13 a	33.60 b	34.22 A
Cultivars averages	V1	30.02 c	32.80 a	30.91 b	31.24 B
	V2	30.08 c	32.72 a	31.24 b	31.35 A
Phosphorus averages	P1	26.06 f	27.58 e	28.00 e	27.21 C
	P2	30.30 d	35.60 a	31.70 c	32.53 B
	P3	33.80 b	35.10 a	33.53 b	34.14 A
aminolevulinic acid averages		30.05 C	32.76 A	31.07 B	

*M1 = 0 mg L-1, M2 = 50 mg L-1, M3 = 100 mg L-1 and P1 = 0 ml L-1, P2 = 1 ml L-1, P3 = 2 ml L-1 and V1 = local cultivar, V2 = Egyptian cultivar.

Total number of leaves per plant (leaf plant-1(

The results of Table 4 indicated a significant effect on the total number of leaves trait when spraying with aminolevulinic acid, as treatment M2 outperformed with a number of leaves of 16.75 leaf plant-1, while the control treatment gave a lower number of leaves of 14.90 leaf plant-1. These results are consistent with (7) in their study of the spinach crop. Spraying with phosphorus had a significant effect on the same trait, as treatment P3 outperformed with the highest number of leaves of 16.83 leaf plant-1, while the control treatment gave the lowest number of leaves of 13.60 leaf plant-1. These results are consistent with (4). As for the cultivars, they did not have a significant effect on the studied trait. The binary interaction between aminolevulinic acid and phosphorus had an effect on the studied trait, as the treatment P2M2 outperformed by recording the highest number of leaves, which reached 18.23 leaf plant-1, while the lowest number of leaves was for the control treatment, which reached 12.90 leaf plant-1. The interaction between aminolevulinic acid and the cultivars had a significant effect on the same trait, as the interaction treatment M2V1 outperformed with the highest number of leaves, which reached 16.76 leaf plant-1, while the lowest number of leaves was recorded for the treatment M1V2, which reached 14.85. The binary interaction between phosphorus and the cultivars for the same trait had a significant effect, as the interaction treatment P3V2 outperformed with the number of leaves, which reached 16.87 leaf plant-1, while the treatment P1V2 gave the lowest number of leaves, which reached 13.53 leaf plant-1. The results of the same table indicated that there was a significant effect of the triple interaction between spraying aminolevulinic acid and phosphorus with the cultivars on the studied trait, as there was superiority for the triple

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interaction treatment P2M2V1, which had a total number of leaves of 18.26 leaf plant-1 and did not differ significantly from the following treatments P2M2V2, P3M2V2, and P3M2V1. The lowest number of leaves was in the triple interaction treatment P1M1V2, which reached 12.83 leaf plant-1 and did not differ significantly from the treatment P1M1V1.

Table 4. Effect of spraying with aminolevulinic acid and phosphorus and their interaction on the total number of leaves per plant (leaf plant-1) for two spinach cultivars (cm(

Cultivars	Phosphorus (ml L^{-1})	aminolevulinic acid (mg L^{-1})			Interaction between cultivars and phosphorus
		M1	M2	M3	
V1	P1	12.96 g	13.96 f	14.10 F	13.67 C
	P2	15.53 de	18.26 a	15.60 De	16.46 B
	P3	16.33 bc	18.06 a	15.96 Cd	16.78 A
V2	P1	12.83 g	13.90 f	13.86 F	13.53 C
	P2	15.23 e	18.20 a	15.86 Cd	16.43 B
	P3	16.50 b	18.13 a	16.00 Cd	16.87 A
Cultivars averages	V1	14.94 c	16.76 a	15.22 B	15.64 A
	V2	14.85 c	16.74 a	15.24 B	15.61 A
Phosphorus averages	P1	12.90 f	13.93 e	13.98 E	
	P2	15.38 d	18.23 a	15.73 C	
	P3	16.41 b	18.10 a	15.98 C	
aminolevulinic acid averages		14.90 C	16.75 A	15.23 B	

*M1 = 0 mg L-1, M2 = 50 mg L-1, M3 = 100 mg L-1 and P1 = 0 ml L-1, P2 = 1 ml L-1, P3 = 2 ml L-1 and V1 = local cultivar, V2 = Egyptian cultivar.

Conclusions

-1aminolevulinic acid at a concentration of 50 mg L-1 is the best and most effective in improving most of the traits.

-2Spraying phosphorus at a concentration of 2 ml L-1 led to improving most of the traits.

-3The binary interaction between aminolevulinic acid at a concentration of 50 mg L-1 and phosphorus at a concentration of 1 ml L-1 gave the best results, and also the local variety V1 was proven to be interacted with 5-aminolevulinic acid at a concentration of 50 mg L-1 to improve most of the traits, while the interaction of phosphorus and the Egyptian variety gave the best results for all the studied traits.

-4The Egyptian cultivar V2 is the best at improving most of the studied traits.

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Recommendations

-1 We suggestion conducting further studies on the use of aminolevulinic acid and its effect on vegetable crops.

-2 We recommend using a combination of aminolevulinic acid at a concentration of 50 mg L-1 and phosphorus at a concentration of 1 mL L-1 to obtain the best results.

-3 Focus on cultivating the Egyptian variety and comparing it with other varieties under the prevailing climatic conditions in Diyala Governorate.

Conflict of Interest

There is no conflict of interest between the authors.

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