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Effect of Humic acid, Cytokinin and Arginine on Growth and Yield Traits of Bean Plant *Phaseolus vulgaris* L. under salt stress

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Abstract

To achieve optimal plant growth and production under salt stress, some products were added in adequate quantities to give a good yield, especially bean plants which are sensitive to salinity. For this purpose, this experiment was carried out during the spring growing season in 2022 in Baghdad, to study the effects of humic acid, cytokinin, arginine and their interaction with 9 parameters that reflect the overall traits of vegetative growth and yield of common bean plants Phaseolus vulgaris L. var. Astraid (from MONARCH seeds, China). The factorial design with 3 replicates was used, each with 7 plants treated via foliar spraying or by addition to the soil. The first factor included three groups; H0, H1 and H2 (0, 6, 12 $Kg.h^{-1}$ Humic acid). The second factor included two groups; C0, and C1 [0, 100 $mg.l^{-1}$ Cytokinins], and the third factor included three groups; A0, A1 and A2 (0, 100, 200 $mg.l^{-1}$ Arginine). During the period of plant growing, furrow irrigation with drip pipe irrigation was used regularly with non-saline water EC= 2.2, during the first growth stage until it reaches the stage of four true leaves then irrigated with well saline water EC=3.4 for the rest of the plant life cycle. As for the treatment with humic acid, the results revealed that H2 treatment caused significantly higher values in most the studied traits. For cytokinin treatment, the results showed that C1 treatment resulted in significantly higher values in most the studied traits. And, there was no significant difference between A0, A1, and A2 except for leaves area, shoot dry weight, and root dry weight which exerts a significant difference between (A1, A2) in comparison to control A0. Based on the results of the binary overlap among treatments, H2C1, H2A2, H2A1 and, triple overlap H2C1A2 and H2C1A1 treatments resulted in the highest values as compared to all other treatments for all the studied traits. In conclusion, the present study comes up with the following: humic acid, cytokinin, arginine, and their interactions enhance most of the plant growth parameters and the pods production of common bean plants under salt stress.

Keywords: Arginine, Bean plant, Cytokinin, Humic acid, Salt stress.

Introduction

The Leguminosae family is economically, environmentally, and, socially important, and it is known as the bean family. It is the third largest family in terms of the number of species after the compound family Asteraceae and the Orchidaceae family. It includes about 770 genera and more than 19,500 species[1, 2]. Legumes were domesticated in different regions of the world since the beginnings of agriculture (about 10,000 years ago)[3], as in common beans in Central America and chickpeas in the Middle East regions, and thus it is considered as one of the most important and richest plant families in terms of diversity because it has a high nutritional value, especially proteins that can reach 40%[4, 5].

Beans are a member of the legume family, which are considered the main food item in most countries of the world, as they have an important nutritional, economic, and industrial impact, and called green beans for their use of green pods bean or dry beans to use dry bean seeds.

Beans are considered one of the warmest season crops, as they are grown in Iraq in two seasons, spring season, starting from March to the end of June, the yield of green pods is harvested at the beginning of May, and the production of dry seeds at the end of June. Autumn is the second season, which begins in late August or early September for green pod production only, whereas, the yield is harvested in October [6]. Harvesting the plant takes place in two stages: 1- The green beans harvesting stage, differs according to the variety, where the pods begin to be harvested 2-3 months after planting and collected 3 to 5 times. 2- The dry beans harvesting stage, the dry beans are collected after 4-5 months until they are dried on the plant, then removed and placed to completely dry under the sunlight.

The common bean belongs to the leguminous family Leguminosae (Fabaceae), subfamily Papilionoidea, genus Phaseolus, and species *P. vulgaris*.

Studying those beans was of great importance, due to their rich nutraceutical values as proteins, (especially albumin, globulin, glutelin, phaseolin, legumin), amino acids (such as isoleucine, leucine, lysine, methionine, phenylalanine, threonine, and valine)[7], and carbohydrates, especially starch[8], It is also rich in vitamins, including thiamine, riboflavin, niacin, pyridoxine, folic acid, ascorbic acids, and retinol [9].In addition, the plant is rich in various macro and microelements such as phosphorus, magnesium, potassium, calcium, iron, zinc, copper, manganese, sodium, and selenium[10, 11], also unsaturated fatty acids such as linoleic and oleic acids, and dietary fiber, such as Pectin[12].

The plant also contains many antioxidants, such as anthocyanins, polyphenols, flavonoids, isoflavonoids, tannins, phytic acid[13, 14].

In a recent study, it was indicated that the accumulation of pharmaceuticals such as Diclofenac have anti-pain and inflammatory diseases such as gout, and mefenamic acid, which is resistant to inflammation and swelling, as well as metronidazole, which has antibacterial activity against anaerobic pathogens[15].

Climate change-related stresses, such as salinity, drought, and heat, reduce the world's crop quantity and quality, leading to significant food, social, and economic insecurity. This is a reality in poor and developing countries, that more than two billion people do not have regular access to safe, nutritious, and sufficient food. According to the National Nutrition Surveys report, 36.9% of the world population suffers from food insecurity[16, 17], and with the world population reaching 10.4 billion in 2080, according to the United Nations estimation,[18] and increasing future expectations of food demand,[19] Therefore, effective measures to increase crop yields must be adopted to overcome the issues of the increase in world population and to mitigate the harmful effects of stresses associated with climate change. This is done through the optimal and effective use of sustainable agricultural practices, especially the addition of some materials that reduce the effect of salt stress resulting from the lack of fresh irrigation water, and the use of saline well water without compromising the quantity and quality of the crop, including leguminous family plants, especially the bean plant, it is very important to integrate into the diet that is important for human health. This is in line with an excellent strategy to achieve the United Nations Sustainable Development Goals for reducing malnutrition and achieving food security[20, 21].

According to the estimation of the Food and Agriculture Organization of the United Nations (FAO) for the year 2021, the cultivated area of the green bean crop in Iraq is 669 hectares, and the production reached 5.9013 tons/ hectare. The cultivated area of dry beans was 760 hectares and the productivity was 6.897 tons/ha.[22], while the cultivated areas of green beans in Iraq witnessed a clear fluctuation during the past years, and this fluctuation in the cultivated areas is due to many reasons, including the problems that agricultural lands suffer from, especially the issues of salinity, such as well as the lack of use practical techniques that lead to raising productivity through production per unit area.

Beans are considered one of the vegetable crops that are sensitive to salinity in the irrigation water, or in the soil. Salinity causes a severe reduction in the yield and quality, because of the rapid increase of saline soil in dry and semi-arid regions of the globe as a result of various climatic changes, insufficient irrigation water, or irrigation with saline-rich water, and widespread soil erosion caused by global warming[23, 24].

The phenomenon of salinity expanded, around 76 million hectares of irrigated lands have problems related to salt stress worldwide[25]. This accounts for 20% of the world's arable land, and an increase in the salinity problem is expected to lead to the loss of up to 50% of arable land by the middle of the 21st century[26].

Salt stress reduced growth factors, water content, and photosynthetic pigments of common bean plants[27], also causing a lot of physiological, phenotypic, and biochemical changes in plants, and greatly affecting plant productivity. Salt-sensitive plants either die, or their productivity decreases after exposure to salt stress. Among the harmful effects of salinity on plants is root growth reduction, burns on the edges of leaves, inhibition of flowering, reduced germination, and seed and yield decline[28, 29, 30].

Humic acid is a product that contains several elements to improve soil fertility and increase the availability of nutrients, thus affecting plant growth and yield, and mitigating the harmful effects of salt stress. It is a substance that is generally formed by the biodegradation of organic matter resulting in a mixture of acids containing carboxylic groups and phenols and has a low molecular weight and high oxygen content[31]. From previous available studies, it was found that the addition of humic acid led to a significant increase in plant length, stem diameter, average leaf area, number of leaves, and dry weight of shoot, as well as the yield of pods, the number of pods in the plant, and the length of the pod, compared to control plants [32] In addition, humic acid was used to mitigate the effect of stress of 8 types of salts (NaCl, Na_2SO_4, $CaCl_2$, $CaSO_4$, KCl, K_2SO_4, $MgCl_2$, and $MgSO_4$) on common beans. It was found that all types of salts led to a negative effect on the weight of the shoot and root, and Chlorine impact was more harmful to shoots, especially as NaCl salt, the addition of humic acid was also reported to have a mitigating effect on all types of salinity, leading to an increase in the internal levels of proline, and increase in the Nitrogen, Phosphorus and total chlorophyll content, and leaf area [33].

Cytokinins influence many aspects of plant growth and development, as recent developments revealed complex physiological functions of cytokinins and discovery of the new function of cytokinins in plant defense systems and stress resistance. Studies have shown that cytokinin content and transmission are reduced due to salinity in different plant species. The decrease in cytokinin level in shoots under stress conditions may be due to suppression of *IPT1,3,5* and/or activation of *CKX1,3,6* in Arabidopsis, a decrease of transport of root cytokinins in xylem, and in the regulation of senescence through chlorophyll degradation, decreased photosynthetic activity, and ultimately cell death[34].

Soil salinity leads to a severe decrease in the availability of water and essential minerals to plants, which hinders growth, but in a study of the role of benzyl adenine (BA) to mitigate the adverse effects of soil salinity in broad bean (*Vicia faba* L), plants were subjected to the stress of 150 mM NaCl, and were Sprayed with BA (0.9 mM), led to improving the growth characteristics of faba bean plants due to an increase in the uptake of K, Ca, and Mg ions, the accumulation of free amino acids, soluble sugars, and soluble proteins, and the activity of some antioxidant enzymes, which indicates that treatment with BA can reprogram metabolic processes to tolerate salinity[35]. In another study to prove the role of cytokinin in mitigating the effects of salt stress on rice plants under salt stress, it was found that the addition of cytokinin improved seed characteristics, including seed yield, the weight of 1000 seeds, and the percentage of seed filling[36].

Many studies on the effects of arginine have mainly focused on increasing abiotic stress tolerance in plants. It was reported that plants treated with arginine improved fruit yield and quality[37, 38]. In another study, it was found that salinity stress causes a decrease of about 17.4% in the number of leaves, but the use of arginine at a rate of 10 and 20 mM showed the improvement of the fresh and dry weights of shoots (gm) recording 20.9%,15.8%, 39.5%, and 31.6%, respectively in comparison to the control. It is interesting to note that the most pronounced effect of the growth parameters studied was found using the higher concentration of arginine[39]. In another study, the addition of arginine increase the vegetative growth length (cm), the weight of a thousand seeds (g), the dry weight of the root (g/plant), the total chlorophyll (μg/cm^2), the percentage of starch, and ash content, as well as the activity of catalase, peroxidase, and ascorbate peroxidase in different concentrations of salts[40].

The present study aimed to evaluate the influence of the addition of humic acid and foliar spray of cytokinin and arginine on the growth and yield of the kidney bean plants under salinity in the local environment, taking into account the problems of deficiency of fresh water, increasing the saline area and growing demand for this plant in the Iraqi market.

Materials and Methods

This experiment was carried out during the spring growing season in 2022 in one of the agricultural fields of Abu Ghraib district, 20 km West of Baghdad governorate, at longitude 44 East, latitude 33 North, and 34.1 m above sea level. A factorial design with three replicates was used. The first factor included three groups; control H0, H1 (6 Kg.h^{-1} Humic acid), and H2 (12 Kg.h^{-1} Humic acid). The second factor included two groups; control C0 (distill water spray), and C1 (100 mg.l^{-1} Cytokinins (benzyl adenine)), and the third factor included three groups; control A0 (distill water spray), A1 (100 mg.l^{-1} Arginine), and A2 (200 mg.l^{-1} Arginine) as a total of 18 treatments, identical to the combinations of humic acid, Cytokinins, and Arginine. Each treatment included 3 biological replicates, and contained 7 plants. The leaves were sprayed with solutions of distilled water containing 0.1 % Tween 20 (polysorbate 20, which is a polysorbate-type nonionic surfactant formed by the ethoxylation of sorbitan) as a surfactant agent.

The cultivation field was divided into three main panels (replicates), and then each panel was divided into 18 secondary panels to represent the experimental unit for each treatment, in a total of three transactions with 54 experimental units. The land was plowed and cleaned off all the growing bushes. Soil samples were taken from the field at a depth of 0-30 cm randomly and mixed well. The sample was analyzed in the Department of Soil Sciences and Water Resources of the College of Agriculture / University of Baghdad to conduct some chemical and physical analyzes of the soil, as in Table 1 and 2.

The experimental unit was represented by 1 m-apart and 3 m-long furrows, each containing 20 plants, and 45 cm apart. During the period of plant growth, furrow irrigation with drip pipe irrigation was used regularly with non-saline water EC=2.2, during the first growth stage until it reaches the stage of four true leaves then irrigated with well saline water EC=3.4 for the rest of the plant life cycle, and weeds were manually kept under control. The broad bean (sensitive to salinity) Phaseolus vulgaris L. Var. Astraid (from MONARCH seeds, China) cultivars, was sterilized and circulated in local agriculture, were sowed in an open field on the first of March 2022. At curds collection (harvest), seven plants were randomly chosen for the experiment. The leaves of plants were sprayed with Cytokinin and Arginine, humic acid was added three times, the first time after the formation of the four leaves, the second time when the flowering began to form, and the third time after two weeks.

The following parameters for growth and yield characteristics were recorded on randomly selected plants: plant height (cm), leaf area (dm^2.plant^{-1}), shoot dry weight (gm), root dry weight (gm), Number of pods (pod. plant^{-1}), pods length (cm), Pod weight (g), Yield per plant (gm plant^{-1}), and Total yield (ton hectare^{-1}).

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For the statistical analysis, the analysis of variance (ANOVA) was performed, the analytical data were analyzed using the statistical program (Genstat Twelfth Edition, 2012), to examine the effects of humic acid, Cytokinin, Arginine, and their interactions on growth and yield of bean plant under salinity stress.

Table 1. Some physical and chemical properties of field soil

Adjective		Value	Unit
Water salinity		3.4	
Soil ph		7.7	
ECe		16.21	Desi Simmons -1
CEC		1.87	Centimoles of a charge / kg soil
ESP		3.48	
Organic Maters		4.0	g/kg soil
gypsum		1.25	
Available ions	nitrogen	63.0	PPM
	phosphorous	14.4	
	potassium	278.0	
Dissolved cations	calcium	12.54	mEq liters -1
	magnesium	9.40	
	potassium	1.02	
	Sodium	2.14	
Dissolved anions	carbonate	219.3	mEq liters -1
	bicarbonate	0.6	
	sulfites	2.87	
	chloride	19.47	
Soil articulations	sand	532	g/kg
	silt	380	
	mud	88	
soil texture grade		Sandy Loam	
bulk density		1.17	megagm m^{-3}
porosity		0. 56	

Table 2. Some chemical properties of ground water used in an experiment

Adjective	EC	Ph	Na	Cl
Value	3.4	7.3	1.31	10.13

Results and Discussion

Plant height

Table 3, demonstrated the results of the effect of the three treatments, the addition of humic acid, spraying of cytokinins (benzyl adenine), spraying of the amino acid (arginine), and their interactions in measuring the height of the bean plant, there was a significant difference in the treatments compared to untreated plants, and the highest value of height plant at the second concentration of humic acid (H2) was 45.3733 cm, the lowest value in the control treatment (H0) was 42.2822 cm. In addition, there was a significant difference in the treatment of cytokinin spraying, as the highest value at the first concentration of cytokinin (C1) was 44.7514 cm, compared to the control treatment, while the lowest value was (C0) 43.5935 cm, whereas, there were no significant differences for the treatment of spraying arginine in comparison to the control treatment.

Similarly, there were no significant differences in the interaction between cytokinin and arginine, whereas, there were clearly significant differences in the interaction between humic and cytokinin. The highest value of treatment with (H1C2) was 45.5000 cm, and the lowest value in the control (H0C0) was 41.1511 cm. Moreover, the interaction of humic and arginine gave the highest value (H2A2) recording 45.5540 cm, and the lowest height (41.8320 cm) was in the control treatment (H0A0).

In regards to the interaction of humic, cytokinin, and arginine, the highest value of (H2C1A2) treatment

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was 45.661 cm, and the lowest value was 40.693 cm for control (H0C0A0).

Table 3. The effect of humic acid, cytokinin, arginine, and their interactions on plant height (cm)

averageA-	A*C	H 2	H 1	H 0	C	A
43.9278 A	43.3458 A	44.987 ABC	44.357 ABC	40.693 F	C 0	A 0
	44.5098 A	45.425 AB	45.133 AB	42.971 CDE	C 1	
44.2669 A	43.6618 A	45.307 AB	44.616 ABC	41.063 EF	C 0	A 1
	44.8720 A	45.413 AB	45.523 AB	43.680 ABCD	C 1	
44.3227 A	43.7729 A	45.447 AB	44.175 ABC	41.697 DEF	C 0	A 2
	44.8724 A	45.661 A	45.367 AB	43.589 BCD	C 1	
0.781 =LSD: A	LSD:A*C= 1.7606	= LSD: A*C*H 2.0597			valueLSD	
---					H x A	
SD: A*H = 1.9865L		45.2060 A	44.7453 A	41.8320 B	A 0	
		45.3600 A	45.0693 A	42.3713 B	A 1	
		45.5540 A	44.7707 A	42.6433 B	A 2	
- averageC					H x C	
43.5935 B		45.2467 AB	44.3827 BC	41.1511 D	C 0	
44.7514 A		45.5000 A	45.3409 AB	43.4133 C	C 1	
= LSD: C 0.6377		LSD: C*H = 1.7606			valueLSD	
---		45.3733 A	44.8618 A	42.2822 B	---	averageH
		= LSD: H 0.781			valueLSD	

For each parameter, treatments followed by the same letter are not significantly different at $p \leq 0.05$.

Leaf Area

The results of Table 4 indicate the effect of treatments on the leaf area of the bean plant, where the highest value at the second concentration of humic acid (H2) and arginine (A2) was 10.11241 dm^2 and 9.50011 dm^2 respectively, and the lowest value for the control treatment (A0) was 9.22689 dm^2. For cytokinin treatment, the highest value at the first concentration (C1) was 9.61546 dm^2, compared to the control treatment (C0) at 9.18755 dm^2.

On the other hand, the results revealed that there were no significant differences in the leaf area index, where a combination of cytokinin and arginine was used Whereas, there were obvious significant differences when the interaction between humic and cytokinin was used, as the highest value in the (H2C1) treatment was 10.2067 dm^2 and the lowest leaf area at (H0C0) treatment was 8.0443 dm^2. Likely, the interaction between humic and arginine gave the highest value (10.1649 dm^2) in the (H2A2) treatment, and the lowest value was 8.1415 dm^2 in the control treatment (H0A0).

Moreover, the threesome interactions of humic, cytokinin, and arginine exert the highest leaf area value (10.2804 dm^2) when treated with (H2C1A2), and the lowest value with control (H0C0A0) was 7.6690 dm^2.

Table 4. The effect of humic acid, cytokinin, arginine, and their interactions on the leaf area (dsm^2)

averageA-	A*C	H 2	H 1	H 0	C	A
9.22689 B	9.0035 A	9.8494 ABCD	9.4921 DEF	7.6690 H	C 0	A 0
	9.4503 A	10.1269 AB	9.6099 CDE	9.4921 DEF	C 1	
9.47752 A	9.2837 A	10.1555 A	9.5344 DEF	8.1611 G	C 0	A 1
	9.6713 A	10.2126 A	9.6814 BCDE	9.1200 F	C 1	
9.50011 A	9.2754 A	10.0495 ABC	9.4741 DEF	8.3027 G	C 0	A 2
	9.7248 A	10.2804 A	9.6261 CDE	9.2678 EF	C 1	
=LSD: A 0.1917	=LSD: A*C 0.7355	= LSD: A*C*H 0.8596			valueLSD	
---					H x A	
= LSD: A*H 0.7644		9.9882 AB	9.5510 B	8.1415 D	A 0	
		10.1841 A	9.6079 B	8.6406 C	A 1	
		10.1649 A	9.5501 B	8.7853 C	A 2	
averageC					H x C	
9.18755 B		10.0182 A	9.5002 B	8.0443 D	C 0	
9.61546 A		10.2067 A	9.6391 B	9.5002 B	C 1	
= LSD: C 0.1566		= LSD: C*H 0.7355			valueLSD	
---		10.11241 A	9.56967 B	8.52244 C	---	averageH
		LSD: H = 0.1917			valueLSD	

For each parameter, treatments followed by the same letter are not significantly different at $p \leq 0.05$.

Dry weight of the shoot

The results of Table 5, showed the effect of treatments on the dry weight of the bean plant shoot, the highest rate was recorded at the second concentration of humic acid (H2) at 33.9444 gm, and the highest rate for the second concentration (A2) of the arginine was 29.2222 gm, and the lowest rate was in the control treatment (A0). It was found that there was a significant difference in the cytokinin treatment, as it reached the highest rate at the first concentration (C1) was 29.6667 gm, compared to the control treatment (C0).

The interaction between cytokinin and arginine revealed that there were no significant differences, while there were obvious significant differences in the interaction between humic and cytokinin, where the highest dry shoot weight in the (H2C1) treatment was 35,000 gm, and the lowest was in the (H0C0) treatment.

Similarly, the interaction between humic and arginine gave a 34,500 gm (H2A2) value, and the lowest was in the control treatment (H0A0).

For the triple interactions, there were significant differences when humic, cytokinin, and arginine were used in combination, as the highest value was 35,000 gm with (H2C1A2) treatment, and the lowest was in the control treatment (H0C0A0)

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Table 5. The effect of humic acid, cytokinin and arginine, and their interactions on dry weight of the shoot g)

averageA-	A*C	H 2	H 1	H 0	C	A
27.5556 B	27.444 A	31.333 BCDE	26.667 HIG	21.333 K	C 0	A 0
	28.667 A	34.667 AB	28.667 EFG	22.667 JK	C 1	
28.7778 AB	27.444 A	33.333 ABCD	26.667 HIG	22.333 JK	C 0	A 1
	30.111 A	35.333 A	30.000 DEFG	25.000 HIJ	C 1	
29.2222 A	28.222 A	34.000 ABC	27.333 FGH	23.333 JKI	C 0	A 2
	30.222 A	35.000 A	30.667 CDEF	25.000 HIJ	C 1	
LSD: A= 1.4337	=D: A*CLS 4.7281	= LSD: A*C*H 5.4764			valueLSD	
---					H x A	
= LSD: A*H 4.8836		33.000 A	27.667 B	22.000 C	A 0	
		34.333 A	28.333 B	23.667 C	A 1	
		34.500 A	29.000 B	24.167 C	A 2	
averageC					H x C	
27.3704 B		32.8889 B	26.8889 D	22.3333 E	C 0	
29.6667 A		35.0000 A	29.7778 C	24.2222 E	C 1	
= LSD: C 1.1706		= LSD: C*H 0.7281			valueLSD	
---		33.9444 A	28.3333 B	23.2778 C	---	averageH
		= LSD: H 1.4337			valueLSD	

For each parameter, treatments followed by the same letter are not significantly different at $p \leq 0.05$.

Dry weight of the root

The results of Table 6, illustrated the effect of treatments on the dry weight of root, the highest value of the average at (H2) was 3.49222 gm compared to control (H0) 2.13889 gm, and there were significant differences. Likewise, the treatments of arginine, exhibits clear significant differences, with the highest rate for (A2) being 2.84056 gm, and the lowest rate for control (A0) being 2.64000 gm. In addition, there was a difference in the cytokinin treatment, as the highest rate at the (C1) was 2.91333 gm, compared to the control treatment (C0) at 2.58074 gm.

While the combination of two treatments, cytokinin, and arginine, there were no obvious significant differences, whereas, the highest value in the (H2C1) treatment was 3.6900 gm, and the lowest in the control treatment (H0C0) was 1.9478 gm, when the combination of humic and cytokinin was used, Similarly, the interaction between humic and arginine, gave higher value (3.6167 gm) for the treatment (H2A2), and a lower value for the control treatment (H0A0) of 2.0033 gm for the dry root weight.

Moreover, there were significant differences in the interaction of humic acid, cytokinin, and arginine, as the highest value was 3.7133 gm with (H2C1A2) treatment, and the lowest value of the triple combination with no-treatment (H0C0A0) was 1.6733 gm.

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Table 6. The effect of humic acid, cytokinin, arginine, and their interactions on the dry weight of root (gm)

averageA-	A*C	H 2	H 1	H 0	C	A
2.64000 B	2.3778 A	3.1167 CD	2.3433 HIG	1.6733 J	C 0	A 0
	2.9022 A	3.7067 A	2.6667 EFG	2.3333 HIG	C 1	
2.76056 AB	2.6100 A	3.2467 BC	2.5433 EFGH	2.0400 I	C 0	A 1
	2.9111 A	3.6500 A	2.7033 EF	2.3800 FGHI	C 1	
2.84056 A	2.7544 A	3.5200 AB	2.6133 EFGH	2.1300 I	C 0	A 2
	2.9267 A	3.7133 A	2.7900 DE	2.2767 HI	C 1	
=LSD: A 0.1418	=LSD: A*C 0.5935	= LSD: A*C*H 0.7518			valueLSD	
---					H x A	
LSD: A*H = 0.6265		3.4117 A	2.5050 BC	2.0033 D	A 0	
		3.4483 A	2.6233 B	2.2100 CD	A 1	
		3.6167 A	2.7017 B	2.2033 CD	A 2	
averageC					H x C	
2.58074 B		2.7200 C	2.5000 D	1.9478 E	C 0	
2.91333 A		3.6900 A	2.7200 C	2.3300 D	C 1	
= LSD: C 0.1158		= LSD: C*H 0.5935			valueLSD	
---		3.49222 A	2.61000 B	2.13889 C	---	averageH
		= LSD: H 0.1418			valueDLS	

For each parameter, treatments followed by the same letter are not significantly different at $p \leq 0.05$.

The number of pods

The results in Table 7, reflected the effect of the three treatments; addition of humic acid, spraying benzyl adenine, spraying of amino acid, and their interactions in measuring the number of pods of bean plants. The highest rate in the number of pods in the second concentration of humic acid was 27.241 and the lowest rate in the control treatment was 20.574., no differences were found in the interaction between cytokinin and arginine.

Unlikely, there were clear differences in the interaction between humic and cytokinin, as the highest number of pods in the (H2C1) treatment was 27,281, and the lowest number of pods in the (H0C0) treatment was 18,504.

Furthermore, the results showed that there was no effect of the treatment of cytokinin and arginine on the average number of pods in the plant. However, the interaction has differed between humic and arginine, where the highest number of pods in the plant was 27.578 in the (H2A2) treatment, and the lowest number of pods with the control treatment (H0A0) was 18.933.

The triple interactions exhibit the highest number of pods in plants treated with (H2C1A2) 27.578, and the lowest number in the non-treated control (H0C0).

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Table 7. The effect of humic acid, cytokinin, arginine, and their interactions on the number of pods

averageA-	A*C	H 2	H 1	H 0	C	A
23.426 A	22.874 A	26.933 A	24.067 AB	17.622 C	C 0	A 0
	23.978 A	26.556 A	25.133 AB	20.244 BC	C 1	
24.637 A	23.407 A	27.222 A	24.889 AB	18.111 C	C 0	A 1
	25.867 A	27.578 A	26.311 AB	23.711 AB	C 1	
24.830 A	23.822 A	27.444 A	24.244 AB	19.778 AB	C 0	A 2
	25.837 A	27.578 A	25.822 A	23.978 AB	C 1	
LSD: A= 2.2965	LSD: A*C= 4.0144	LSD: A*C*H =5.4592			valueLSD	
---					H x A	
LSD: A*H =4.8139		26.744 A	24.600 ABC	18.933 D	A 0	
		27.400 A	25.600 AB	20.911 CD	A 1	
		27.578 A	25.033 AB	21.878 BCD	A 2	
averageC					H x C	
23.3679 A		27.200 AB	24.400 BC	18.504 D	C 0	
25.2272 A		27.281 A	25.756 AB	22.644 C	C 1	
LSD: C = 1.8751		LSD: C*H =4.0144			valueLSD	
---		27.241 A	25.078 A	20.574 B	---	averageH
		LSD: H =2.2965			valueLSD	

For each parameter, treatments followed by the same letter are not significantly different at $p \leq 0.05$.

Pods length

The findings of Table 8, showed the effect of treatment on measuring the length of pods in bean plants, it was found that there were no significant differences in the average of humic acid, cytokinin, and arginine treatment on the length of pods.

Likely, no differences were observed in the interaction of arginine and cytokinin, and the interaction between humic and cytokinin. However, there was a significant effect of the interaction between humic and arginine, where the highest value in the (H2A0) treatment was 12.1167 cm, and the lowest value in the (H0A0) treatment was 11.1722 cm.

Interestingly, the highest value was found when a combination of all treatments was conducted (H2C1A2), and the lowest value was in no-treated control (H0C0A0).

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Table 8. The effect of humic acid, cytokinin, and arginine, and their interactions on the pod length (cm)

averageA-	A*C	H 2	H 1	H 0	C	A
11.7981 A	11.6074	11.8556 A	11.7444 A	11.2222 AB	C 0	A 0
	11.9889 A	12.3778 A	12.4667 A	11.1222 AB	C 1	
11.6259 A	11.4667 A	11.4778 AB	11.5222 A	11.4000 AB	C 0	A 1
	11.7852 A	11.7000 A	11.2000 AB	12.4556 A	C 1	
11.3037 A	11.4148 A	11.1556 AB	11.1667 AB	11.9222 A	C 0	A 2
	11.1926 A	12.0444 A	11.4444 AB	10.0889 B	C 1	
LSD: A= 0.5815	LSD:A*C= 0.8683	LSD: A*C*H =1.4235			valueLSD	
---					H x A	
LSD: A*H =1.0408		12.1167 A	12.1056 A	11.1722 AB	A 0	
		11.5889 AB	11.3611 AB	11.9278 AB	A 1	
		11.6000 AB	11.3056 BA	11.0056 B	A 2	
averageC					H x C	
11.4963 A		11.4963 A	11.4778 A	11.5148 A	C 0	
11.6556 A		12.0407 A	11.7037 A	11.2222 A	C 1	
LSD: C =0.4748		LSD: C*H = 0.8683			valueLSD	
---		11.7685 A	11.5907 A	11.3685 A	---	averageH
		LSD: H = 0.5815			valueLSD	

For each parameter, treatments followed by the same letter are not significantly different at $p \leq 0.05$.

Pod weight average

The results of Table 9, revealed that there was a significant effect on plants treated with the treatments, where the highest rate of pod weight in the plants was 4.30557 gm at (2H), and the lowest average pod weight with the control treatment (H0) was 3.69496 gm. It was found that the cytokinin spraying treatment had a significant difference, as the highest rate with (C1) was 4.10010 gm in comparison to the control treatment, where the lowest rate at (C0) was 3.94772 gm. Whereas, there were no significant differences in arginine treatments compared to the control, Similar to the interaction between cytokinin and arginine.

Unlikely, the results showed that there were differences in the interaction between humic and cytokinin, was the highest value in the treated plant (H2C1) was 80.2594 gm, and the lowest value in the untreated plant (H0C0) was 78.3580 gm. The same with the interaction between humic and arginine, there were significant differences between the interactions, as the highest value in (H2A1) was 4.3459 gm, and the lowest value in the control (H0A0) was 3.6568 gm.

In regards to the threesome interactions of humic, cytokinin, and arginine, the highest value was 4.4343 gm when treated with (H2C1A2), and the lowest value was 3.4943gm when the control treatment (H0C0A0) was used.

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Table 9. The effect of humic acid, cytokinin and arginine, and their interactions on the weight of the pod (gm)

averageA-	A*C	H 2	H 1	H 0	C	A
3.98559 A	3.8705 A	4.2074 ABCD	3.9097 DEFG	3.4943 H	C 0	A 0
	4.1007 A	4.2857 AB	4.1970 CDE	3.8194 FGH	C 1	
4.02287 A	3.9670 A	4.3119 ABC	3.9522 CDEF	3.6368 DEFG	C 0	A 1
	4.0788 A	4.3799 AB	4.1246 ABCDE	3.7319 FGH	C 1	
4.06327 A	4.0057 A	4.2142 ABCD	4.0603 BCDEF	3.7428 FGH	C 0	2 A
	4.1208 A	4.4343 A	4.1834 ABCDE	3.7447 FGH	C 1	
LSD: A= 0.1487	LSD: A*C= 0.3161	LSD: A*C*H = 0.3701			valueLSD	
---					H x A	
LSD: A*H =0.360		4.2466 AB	4.0534 B	3.6568 C	A 0	
		4.3459 A	4.0384 B	3.6843 C	A 1	
		4.3242 A	18 4.12 AB	3.7437 C	A 2	
averageC					H x C	
3.94772 B		80.1084 A	79.7438 AB	78.3580 C	C 0	
4.10010 A		80.2594 A	80.0234 AB	79.3421 A	C 1	
LSD: C = 0.1214		LSD: C*H =0.3161			valueLSD	
---		4.30557 A	4.07119 B	3.69496 C	---	averageH
		LSD: H = 0.1487			valueLSD	

For each parameter, treatments followed by the same letter are not significantly different at $p \leq 0.05$.

Yield per plant

The results of Table 10 indicated a crucial role of humic treatment in terms of the yield of the plant, as the highest mean of plant yield was 117.139 gm when the treatment (2H) is used. While the treatment of arginine didn't exert any significant differences in the yield per plant. It was observed that the cytokinin treatment excelled, as the highest rate of yield per plant was 103.739 g in treatment (C1), and the lowest rate of yield per plant was 93.051 gm in the control treatment (C0).

Whereas there were no differences in the interaction between the treatments of arginine and cytokinin in the experiment in terms of the yield of one plant. Otherwise, the interaction between humic and arginine, gave the best value of yield per plant in the treatment (H2A2), and the lowest value was in the control treatment (H0A0).

In the same context, with the interaction between humic and cytokinin, the highest value was obtained in the (H2C1) treatment, and the lowest value was in the control treatment (H0C0).

In consideration of using threesome interactions, there were very significant differences between the overlapping treatments for the yield per plant, the highest value was with (H2C1A2) treatment, and the lowest value was with no-treatment control (H0C0A0).

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Table 10. The effect of humic acid, cytokinin and arginine, and their interactions on the yield of one plant (gm)

averageA-	A*C	H 2	H 1	H 0	C	A
94.275 A	89.65 A	113.27 ABC	94.12 CDEF	61.57 G	C 0	A 0
	98.90 A	113.67 ABC	105.49 ABCD	77.54 EFG	C 1	
99.545 A	93.39 A	117.33 AB	97.00 BCDE	65.82 G	C 0	A 1
	105.70 A	120.13 A	108.35 ABCD	88.62 DEF	C 1	
101.365 A	96.11 A	115.60 AB	98.67 BCD	74.07 FG	C 0	A 2
	106.62 A	122.83 A	107.25 ABCD	89.77 DEF	C 1	
LSD: A= 8.7708	LSD: A*C= 20.211	= LSD: A*C*H 22.939			valueLSD	
---					H x A	
LSD: A*H = 21.384		113.468 AB	99.803 B	69.555 C	A 0	
		118.733 A	102.677 B	77.223 C	A 1	
		119.216 A	102.958 B	81.921 C	A 2	
averageC					H x C	
93.051 B		115.401 AB	96.597 C	67.155 E	C 0	
103.739 A		118.877 A	107.029 BC	85.311 D	C 1	
LSD: C =7.1613		LSD: C*H =20.211			valueLSD	
---		117.139 A	101.813 B	76.233 C	---	averageH
		LSD: H = 8.7708			valueLSD	

For each parameter, treatments followed by the same letter are not significantly different at $p \leq 0.05$.

Discussion

The outcomes of the present study can be initially discussed in the light of affecting the addition of humic acid, spraying of cytokinins (benzyl adenine), spraying of an amino acid (arginine), and their interactions on the bean plant Phaseolus vulgaris L, to tolerate salinity stress via mitigating the salinity damage and reducing its toxicity for the plant by a significant increase in the vegetative growth traits, i.e. plant height, leaf area, fresh and dry weight for both shoot and root, and yield.

Salt stress is one of the main environmental factors that weaken physiological processes in plants, including vegetative and flowering growth factors and water content,[27] by reducing the metabolism such as photosynthesis efficiency, decomposition of pigments, and vacuole function[41]. As well as deterioration of the chloroplast membrane, and other organelles such as mitochondria and endoplasmic reticulum[42], disruption of plant hormones function, like auxin IAA, alteration of basic metabolic pathways, and manipulation of gene expression pattern[43]. Salinity stress leads to an ionic imbalance, which leads to the accumulation of harmful ions in plants, such as Na and Cl,[44] and reducing the absorption of macro elements such as N, P, K, Ca, and Mg,[45, 46] and microelements such as B, Zn, Cu and Fe[47]. It leads to an increase in the production of reactive oxygen species (ROS), as it causes cellular imbalance, which mainly results in cell membrane damage, and deteriorates biomolecules such as lipids, DNA, and proteins[42, 48].

The significant reduction of salinity damage and stress tolerance resulting from the addition of humic acid to the plant leads to improve the vegetative growth characters[49] by enhancing the absorption of important nutrients, and increasing their transfer and then their accumulation in the shoot system in a significant way, such as macronutrients N, P, K, Ca, Mg, S, and micronutrients such as Fe, Zn, Mn and Cu [50], As well as reducing the accumulation of some toxic elements by reducing their uptake, such as Na [51, 52], as well as elongating root cells, increasing oxygen uptake and respiration, and increasing chlorophyll pigments and thus the efficiency of photosynthesis, which leads to plant tolerance to high concentrations of salinity[53, 54].

It was found that spraying the growth regulator cytokinin mitigates the toxicity of saline water, perhaps through vegetation growth parameters, and also reduced lipid peroxidation, improved oxidative defense in leaves, and increases membrane permeability, as well as substitutes the oxidative damage by enhancing antioxidant defense mechanisms such as increasing the enzymatic activity of superoxide, catalase, peroxidase, ascorbate peroxidase, and scavenging ROS[55, 56] Cytokinins also regulate the ability of plants to absorb many nutrients from the environment, including nitrogen, phosphorous, sulfur and iron[57], which regulates mineral balance. The nutrient status of plants regulates plant growth[58]. Positive regulation of substances exerts protection against osmotic stress and ionic balance, antioxidant activity, and finally plant growth and yield[55].

Amino acids can be used as neutralizing compounds against stress conditions, and as a useful strategy to mitigate salt stress, as the foliar spraying of arginine leads to protective effects on plants in alleviating salinity stress by both improving vegetative growth qualities, and improving the enzymatic activities of antioxidant enzymes. Catalase, Peroxidase, Superoxide Dismutase, and Ascorbate Peroxidase[59, 60], and the increase in phenolic substances and osmotic modification, which lead to a better antioxidant defense system for the plant and osmolytes accumulation[44, 61]. When using arginine, the pigments of photosynthesis increased significantly in plants treated with arginine under salinity stress, and thus the efficiency of the photosynthesis process increased, as a result, carbohydrates accumulated, which formed the basic framework for the plants[62, 63]. In addition, free amino acids were increased, as well as a significant decrease in sodium, while a significant increase was found in potassium and phosphorus, and a non-significant increase in nitrogen, calcium, and magnesium, which leads to a significant increase in yield characteristics.[40, 45]. Thus, the ratio of K^+ / Na^+ increased in the leaves, as increased K^+ absorption helps to maintain ionic balance, regulate osmotic balance, maintain swelling, and regulate membrane potential[64, 65].

Conclusion

In conclusion, the present study concluded the following: humic acid, cytokinin, arginine and their interactions enhance the growth and production of bean plants under salt stress. The optimum yield of bean plants under salinity was obtained when 12 $Kg.h^{-1}$humic acids was applied, in the overlapping treatment (H2C1A2) (12 $Kg.h^{1}$ Humic acid), (100 $mg.l^{-1}$ Cytokinins (benzel adenine)) and (200 $mg.l^{-1}$ Arginine) concentration for the local agricultural climate.

Authors' Declaration

- Conflicts of Interest: None.
- We hereby confirm that all Tables in the manuscript are ours.
- Ethical Clearance: The project was approved by the local ethical committee in University of Baghdad.

Authors' Contribution Statement

M.K. designed the study. T.S. performed this work, T.S. and M.K. contribute to writing the manuscript. M.K. read and approved the final manuscript.

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تأثير حامض الهيوميك والسايتوكينين والأرجينين على صفات النمو وحاصل نبات الفاصوليا *Phaseolus vulgaris* L تحت الإجهاد الملحي

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الخلاصة

من أجل تحقيق النمو والإنتاج الأمثل للنبات في ظل الإجهاد الملحي، تمت إضافة بعض المنتجات بكميات كافية لإعطاء محصول جيد، وخاصة نباتات الفاصوليا الحساسة للملوحة. لهذا الغرض نفذت هذه التجربة خلال موسم النمو الربيعي 2022 في احد الحقول الزراعية في قضاء ابو غريب بمحافظة بغداد لدراسة تاثير حامض الهيوميك والسايتوكينين والارجينين وتداخلاتهم على صفات النمو والحاصل الخضري لنباتات الفاصوليا *Phaseolus vulgaris* L. (بذور صنف أسترايد من MONARCH الصين)، تم استخدام تصميم عاملي بثلاث مكررات، كل منها يحتوي على 7 نباتات تمت معاملتها بالرش الورقي اوإضافتها إلى التربة. العامل الأول شمل ثلاث مجاميع؛ H0 السيطرة، H1 (6 كغم/هكتار حامض الهيوميك)، وH2 (6 كغم/هكتار حامض الهيوميك)، العامل الثاني شمل مجموعتين؛ C0 السيطرة (رش الماء المقطر)، و C1 (100 ملغم / لتر السايتوكينين (بنزل أدينين))، والعامل الثالث شمل ثلاث مجاميع؛ A0 السيطرة (رش الماء المقطر)، A1 (100 ملغم / لتر ارجنين)، A2 (200 ملغم / لتر ارجنين)، خلال فترة نمو النبات، تم استخدام الري بالتنقيط بانتظام بالماء غير المالح EC = 2.2 ، خلال مرحلة النمو الأولى حتى الوصول إلى مرحلة أربع أوراق حقيقية، ثم تم اكمال الري بمياه البئر المالحة EC = 3.4 لبقية دورة حياة النبات. أما بالنسبة لاضافة حامض الهيوميك، فقد أوضحت النتائج أن معاملة H2 تسببت في أعلى قيم معنوية في جميع الصفات المدروسة، باستثناء ارتفاع النبات وعدد القرون وطول القرون حيث لم يكن هناك فرق معنوي بين H1 وH2 ، فيما يتعلق بالرش بالسايتوكينين، أوضحت النتائج أن معاملة C1 أعطت أعلى قيم معنوية في جميع الصفات المدروسة، باستثناء عدد وطول القرنات، حيث لم يكن هناك فرق معنوي بين C0 وC1، كما كشفت النتائج أنه لا يوجد فرق معنوي بين A0 و A1 و A2 باستثناء مساحة الورقة والوزن الجاف للمجموع الخضري والجذري والتي وجدت فرقاً معنوياً بينA1 وA2 ومعاملة السيطرة A0، وبناءً على نتائج التداخل الثنائي بين المعاملات نتج عن معاملات H2C1 وH2A2 و H2A1 والتداخل الثلاثي H2C1A2 وH2C1A1 أعلى القيم مقارنة بجميع المعاملات الأخرى لجميع الصفات المدروسة. النتائج المقدمة هنا قد تسهل تحسين زراعة الفاصوليا مع تراكيز المنتجات المناسبة للزراعة المستدامة، في الختام توصلت الدراسة الحالية إلى ما يلي: حامض الهيوميك والسايتوكينين والأرجينين وتداخلاتهم يعزز بشكل كبير بعض صفات النمو وإنتاج قرون نباتات الفاصوليا تحت الاجهاد الملحي.

الكلمات المفتاحية: الارجنين، الفاصوليا، السايتوكاينين، حامض الهيوميك، الاجهاد الملحي.