The influence of foliar splatter with the amino acid arginine on some physiological characteristics of cowpea plant *Vigna unguiculatal* L. exposed to salt stress

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Abstract

The experience was conducted utilizing pots according to the completely random design method (RCBD) during the cultivation period 2022-2023 to study The influence of splatter by arginine and from condensations (0.50.100) Mg. L^{-1} and irrigating with saltine NaCl and in condensations (0.5.10.15) $ds.m^{-1}$ on some physiological adjectives of cowpea plants The outcoms indicated important increasing in the content untill vitamin C in the plant, Proline, H_2O_2, Salicylic acid at the treatment with the same concentration from NaCl, especially at the concentration of 15 $ds.m^{-1}$ While there was important decreases in the activity of the enzyme catalase in this concentration from NaCl, when spraying the plant with the arginine lead to signification increased in the activity of the enzyme catalase and the content of vitamin C in the plant and Salicylic acid while there was a significant decreases in the Proline, H_2O_2 on especially at the concentration of 100 mg. L^{-1} of arginine even for over loop have an effect on the physiological characteristics.

Keywords: foliar splatter, amino acid arginine, cowpea plant, *Vigna unguiculatal* L., salt stress

Introduction

Cowpea is a leguminous crop , which are of important nutritional and economic importance, as they contain many essential elements, including iron, sodium, potassium and zinc. As well as containing fiber and folic acid (Heuze *etal.*, 2015). Cowpea cultivation spreads in most country for the old ancient counters like Egypt it is considered a basic food crop represented by its green pods or dry seeds, or it is added as fertilizer to the soil or green fodder for animals, in addition to its role in maintaining the integrity of the sensory organs, including the nervous, digestive and circulatory systems, regulating blood sugar and skin safety(El-Shaieny etal., 2015). The amino acid arginine is one of the amino acids Which is not synthesized inside the body, so it must be obtained from foods rich in arginine, such as dairy products to the arginine important role in activation of plant growing and increasing The rate of cell divisions, maintaining the water content of the plant, and increasing the plant's immunity and vitality to resist environmental stresses by regulating the metabolic processes inside the plant. (Winter etal., 2015; Xia etal., 2014 ; Conceicao etal. 2021)

That salt stress a specific effect on the growth and productivity of crops by irrigating plants with saline water or as outcom of the gathering of salts from the soil for long periods as a result of natural processes Salts have an effect on plant growth and productivity because they increase the conditions of oxidative stress in the presence of free roots formed inside plant cells in addition to the effect of these salts on the internal level of the plant by hormones and internal growth regulators, which unfavorable influence the vegetative evolution average of the plant (Abd El-Wahab.,2006 ; Taffuo etal., 2010; Cirillo etal., 2016).

Materials and working methods

The experiment was conducted for the cultivation period 2022-2023 According to the Randomized Complete Block Design (RCBD) as an integrated experiment (4X3) and with three replications; the experiment included the following factors:

1- Four concentrations of solution NaCl and they are (0,5, 10,15) $ds.m^{-1}$ or its equivalent in M $mol.L^{-1}$ which is (0,50,100,150)M mol. L^{-1}

2- Three concentrations of arginine (0,50,100) mg. L^{-1}

The experiment consisted of three replicates and each replicate contained12 pots if the number of pots in Experience 36 pots.

Cowpea seeds were sown on 15/3/2022 and at an average of three lines And that for cultivation and with four holes, the depth of one hole reached 2 cm if the potting soil was analyzed before planting in the central laboratory subordinate College of Agricultural Engineering Sciences. University of Baghdad to know some of its chemical and physical properties Table1.

Table (1): Some chemical and physical properties of soil

Adjective	The value
Phosphorous	5.50ppm
Nitrogen	60 ppm
Potassium	180ppm
Electrical conductivity(EC)	2.25 $ds.m^{-1}$
soil ratio	mixture – clay
PH	7.6

When the plant reaches the stage 3-4 papers dated 5/4/2022 were sprayed With the amino acid arginine by hand sprayer and in the early morning by taking 1g of

this acid and dissolve it in a small amount of dripped water, later complete the size 1000 ml with dripped water, later the arginine concentrations were prepared according to the dilution law $C_1V_1 = C_2V_2$
As for the saltine solution NaCl the plant were irrigated with it after 10 days of spraying them with the amino acid arginine, on 15/4/2022. It was prepared by preparing a stock solution of this salt at a concentration of 1 molar, from which the saltine concentrations were prepared according to the law of dilution, and the perfusion process for plants continued on a regular basis.

Study trait

1- **Physiological traits**

The physiological characteristics of the plants were measured by date 20/5/2022 which are as follows:

A- **Catalase enzyme activity (absorption unit .ml $^{-1}$)**

where has been mashed1gm From fresh weight leaves are added to it10 ml of K_2HPO_4 (0.1m) mixture , later the patterns were filtered by pieces of gauze and located in a centrifuge at 4°C. for 30 min at a speed of 1000 (rev. min $^{-1}$). Then the solutions were prepared as follows:

Solution A: It was prepared by dissolving 1.8 gm of K_2HPO_4 in an amount of dripped water also later completing the size to 200 ml with dripped water.

Solution B: It was arranged by dissolving 1.3 g of K_2HPO_4 in an amount of dripped water also later completing the size to 200 ml with dripped water.

Solution K_2HPO_4 (50) mol: This solution is prepared from a certain volume of solution B, to which solution A is added (50 ml) up to PH = 7

solution H_2O_2: Prepare this solution by taking 0.3 of H_2O_2 (30%) and fill the volume to 100 ml of K_2HPO_4. Taking 0.1ml of the sample was added to it 1 ml of H_2O_2 solution and 1.5ml of buffer solution and mixed well and the absorbance readings were measured by a Uv.Spectrophotometer and at 240nm wavelength. The change in absorbance was followed every 30 seconds and for 3 minutes after which the stimulant activity was estimated according to Aebi (1974). According to the following equation:

Catalase enzyme activity = (reading deviceΔ/ time /0.1X0.01) sample volume ml 0.01 , the quantity of stimulant that generated raised light assimilation .

B- Vitamin C content (mg . 100 g^{-1} fresh weighing) in the plant leaves

1gm of fresh weight plant bracts were dissolved in10ml of Oxalic acid (0.05M) solution and was placed in the shade as all sunlight , then the samples were filtered. After that, the following mixture were prepared:

Solution A: It was prepared by dissolving 5gm of Ammonium molybdate in 100ml of distilled water.

Solution B: It was prepared by taking a weight of Oxalic acid (0.05m) according to the molarity law and mixing it with EDTA compound $C_{10}H_{16}N_2O$ (0.02M) following the same chemical equation also later completing the size to 100 ml with dripped water.

Solution C: Prepare by taking 5ml of H_2SO_4 and adding to 100ml of distilled water.

Solution D: Prepared by dissolving 5gm of Meta phosphoric acid + Acetic acid in 100 ml of dripped water and then withdrawal 30 of this solution also mix by 80 ml of Acetic acid and bring the volume up to 500ml with dripped water.

then take 2 ml of the filtrate was added to it 2.5 ml of solution B, 1 ml of mixture C, 2 ml of mixture A also 0.5 ml of mixture D and completed the size to 25 ml with dripped water, then read the absorbance B apparatus Spectrophotometer at wavelength 760 nm Then the measurement curve was prepared and the relationship between the concentrations of vitamin C and the absorbance values for each concentration of this vitamin was plotted according to the method mentioned (Hussain etal. 2010).

C-Proline acid content (µg. g $^{-1}$fresh weighing) in plant bract

Were taken 0.5gm of fresh weight plant leaves and put them in a bowl and add 10ml of sulfosalicic acid to it. Then the samples were separated by centrifuge at a fast of 1000 rounds. Min^{-1} and for 10 minutes, then take 2 ml of the filtrate and add to it 2 ml of glacial acetic acid and 2 ml of tetrahydrin mixture , later leave the solution at little warmth of the yellow pigment appears, later place the cylinders in a water bath For for 30 minutes , after cooling the patterns , add to it 4ml of toluene and then 3ml of the top layer of samples were withdrawn and measured with a device. Visible Spectrophotometer At 520nm wavelength, estimate the concentration of proline from plant leaves according to the method mentioned by Bates etal (1973). According to the following equation:

proline = (reading X 20/ sample weight) X 1.47

D-The hydrogen peroxide content of the leaves (Mmol. g^{-1} fresh weight)

Taking 1 gm of plant leaves and 2 ml of Trichloroacetic acid were added to it, then the samples were filtered and the filtrate distinct with centrifuge for 15 moments from a fast of 1200 rounds . Min^{-1}after that, the solutions came to my agencies:

Solution A: It was prepared by dissolving 0.3 g of K_2HPO_4 in an amount of dripped water also later supplemented to 200 ml of dripped water.

Solution B: It was arranged by dissolving 0.2 of K_2HPO_4 in an amount of dripped water also later supplemented to 200 ml of dripped water.

mixture K_2HPO_4 (0.010M): It was arranged with inserting a determine volume of mixtur B to 200 ml of solution A down to PH = 7.

mixture Potassium Iodide (1M): It was arranged by dissolving 3.20 ml of KI in an amount of distilled water and then supplemented with 200 ml of dripped water.

Solution H_2O_2 (0.010M): It was prepared by dissolving 0.17 ml of H_2O_2 in an amount of distilled water and then supplemented to 200 ml of distilled water.

Taking 0.5 ml of the filtrate was incerted to it 0.5 ml of K_2HPO_4 solution and 1 ml of KI solution, then the test tubes were placed with a spectrophotometer and at the wavelength 390 nm, then 0.5 ml of each dilute solution (9.7.3.1.0.5) µmol $^{-1}$ ml was taken and added to a

substance The reaction was measured by a Spectrophotometer at a wavelength of 390nm, then the measurement curve H_2O_2 was drawn, which corresponds to the absorbance of the samples, and then the content of the samples was estimated from H_2O_2 according to the method mentioned by Veli kova etal (2000).

E- Salicylic acid content (µg. ml^{-1}) of the papers vegetarian

Taking1 gm of fresh weight leaves were placed in the freezer for 24 hours, then mashed and 100 mg of samples were taken and placed in test tubes, then distinct with a centrifuge as 10 moments from a fast untill 1000 rounds. Minute $^{-1}$ Then 100 µl was withdrawn and 3ml of FeCl3 was added to it until the appearance of the violet color, after which the absorbance was read B Spectrophotometer at 540nm wavelength and then make the standard curve According to the concentration of heatz salicylic acid by comparing the absorbance of each concentration with the measurement curve and according to the method presented Warrier et al (2013).

statistical analysis

The studied traits were numerically analyzed inrelation to the randomized complete block form method(RCBD) also comparing the important differences between the arithmetic means using the least significant difference between the means also at the possibility limit of 0.05 (SAS.2012).

outcomes and debates

Chart (2) outcoms indicated there is an important decrease in the average activity of the enzyme catalase while treating the plant with NaCl and in particular condensation 15 $ds.m^{-1}$ these allow the lowest average for the trait reached 42.92 (absorption unit. ml $^{-1}$) Compared to control and extreme 60.36 (absorption unit. ml^{-1}) with a percentage decrease of 28.89% due to the stressful conditions formed by the action of Na^+ and Cl^- ions, which are Increases from the exposure of the plant to free roots that destroy cell membranes and inhibit the activity and effectiveness of enzymatic antioxidants (Mohammed and Al-Ubaidy., 2020). The chart also indicated an important raised in the middle of the trait when treating the plant with Arginine in particular concentrate 100 $mg.L^{-1}$, which allow the upper average of 54.28 (absorption unit. ml^{-1}) compared to the control, which amounted to 3274 (absorption unit. ml^{-1}). With a percentage increase of 65.79% because arginine plays an important role in activating vital processes within the plant and by providing it Nitrogen component in the construction of CO-enzyme, prophyrins, which supplies the plant with the energy needed for vital processes and activates the plant's internal defense mechanism against oxidative conditions by the action of salt ions (Hassan etal., 2010) and this is consistent with what was stated Abdullah (2020) on the tomato. The interference has an important influence on that subjects if the concentration is given zero $ds.m^{-1}$ from NaCl and 100 $mg.L^{-1}$ of arginine were higher than the average, reaching 82.75 (absorption $unit.ml^{-1}$), while the lowest average was 32.74 (absorption $unit.ml^{-1}$) when condencation 15 $ds.m^{-1}$ from NaCl and zero $mg.L^{-1}$ of arginine

chart(2): effect of foliar spraying with arginine on the average of catalytic enzyme activity (absorption unit.ml^{-1}) of cowpea plants exposed to salt stress.

The result of table(3) indicate to significant increasing in the of average vitamin C content

sodium chloride Concentrations (ds.m^{-1})	**Arginine concentrations (mg.L^{-1})**			
	0	**50**	**100**	**Sodium chloride**
0	**42.92**	**55.41**	**82.75**	**60.36**
5	**37.63**	**43.61**	**60.50**	**47.25**
10	**35.46**	**41.96**	**62.47**	**46.63**
15	**32.74**	**41.74**	**54.28**	**42.92**
Arginine average	**37.19**	**45.68**	**65.00**	
LSD (0.05)		**arginine=2.25**	**Sodium chloride=2.60**	**overlap=4.51**

when treating the plant with NaCl, especially the condensation 15 ds.m^{-1} which allow the highest average 200.37 (mg.100g $^{-1}$ fresh weight) compared to control of 100.95 (mg.100 g $^{-1}$fresh weight) and in a percentage increase estimated 98.48% This is due to the increase in the activity of free roots, including OH^- and O^{2-} roots formed by the action of salt ions, these possess a negative influence on the structure of cell membranes, causing their destruction. This prompted the plant to activate its defense mechanism to protect its cellular parts, especially by increasing the effectiveness of some enzymes, including an enzyme (GDP-D-mannose pyrophosorylase), which converts glucose sugar into vitamin C (wang etal,.2018) and that in line with what Al-Saady (2019) brought on the mung plant.

The chart also point out that there was an important raise in the average of that trait when treating the plant with arginine, especially the condensation 100 mg.L^{-1}, which allow the highest middle for the trait, was 179.46(mg.100g $^{-1}$ fresh weight) compared to control of 101.21 (mg.100g $^{-1}$ fresh weight) with a percentage increase of 77.31% because arginine is one of the amino acids that prepare the plant with the necessary elements for its growth, including the important nitrogen element in increasing the content of the leaves of non-enzymatic antioxidants, including vitamin C (Koota.,2011).

The presence of amino acids effectively contributes to the activation of vital activities because they contain the

necessary C and N elements in building proteins and the chlorophyll molecule and maintaining cellular structures from the various stresses to which the plant is exposed, thus activating its defense mechanism, including its content of vitamin C (Conceicao etal.,2021). The interaction also has an important influence if the condensation of 10 $ds.m^{-1}$ is given from NaCl and 100 $mg.L^{-1}$ of arginine had the highest middle for the trait reached 214.79 (mg. $100g^{-1}$fresh weight) compared to the lowest mean for the trait was 45.18 (mg. $100g^{-1}$fresh weight) at a concentration of 10 $ds.m^{-1}$ from NaCl and zero $mg.L^{-1}$ of arginine.

Chart (3) : influnce of foliar splatter with arginine on the average content of vitamin C (mg. 100 g^{-1} fresh weight) of cowpea plant exposed to salt stress.

Concentrations sodium chloride($ds.m^{-1}$)	**Arginine concentrations ($mg.L^{-1}$)**			
	0	**50**	**100**	**Sodium Chloride average**
0	60.95	**104.33**	**137.57**	100.95
5	90.07	**120.15**	**153.09**	121.10
10	45.18	**150.23**	**214.79**	136.73
15	208.64	**180.10**	**212.37**	200.37
Arginine average	101.21	**138.71**	**179.46**	
LSD (0.05)		**Arginine=1.72**	**Sodium chloride=1.99**	**overlap= 3.44**

The results of Table (4) showed that there was a significant increase in the average proline content when the plant was treated with NaCl, especially the condensation 15 $ds.m^{-1}$, which gave the highest middle for the character amounting to 41.07 (µg $.g^{-1}$ fresh weight) compared to the control, which amounted to 28.11 (µg.g^{-1} fresh weight) with a percentage increase of 46.01% that increasing the condensation of this acid is a reaction to preserve the cellular parts from the negative effects of salt stress, so the concentration of proline increases in the cytoplasm of plant cells to maintain its stability in the cell membranes and the effectiveness of some enzymes as a defense method to withstand salt stress (Al-Harby etal.,2018).

Salt stress stimulates the plant to build substances that regulate cellular responses and increase the proportion of energy compounds, including NADP, given that salinity plays a role in the speed of proline building by inhibiting its

oxidation and activating proline-building enzymes (Gumi etal.,2013). This is in keeping with Singh and Dwivedi (2018) on the pea plant.

The outcomes of the chart also pointout that there was an important decrease in the average of this trait when the plant was treated with arginine, especially the condensation of 100 mg. L^{-1}, which gave the lowest average of 30.86 (µg. g^{-1} fresh weight) compared to the control, which amounted to 37.66 (µg. g^{-1} fresh weight), with a percentage decrease of 18.05%. This is due to the fact that arginine plays a positive and effective role for the plant in providing the important nitrogen element in Synthesis of cellular components and stimulation of protein synthesis and preservation of cell membranes from cellular destruction by oxidative conditions (Winter etal.,2015). Stimulating the average of vegetal and root development of the plant and activating its defense mechanism from any free roots formed by stress, including proline acid, which activates as a natural reaction to increase the plant's tolerance to Na+. Cl^{-}. The interaction also had a significant effect on this trait, especially the condensation of 15 $ds.m^{-1}$ of NaCl and zero mg. L^{-1} of arginine, which gave the highest mean for the trait that reached 46.47 (µg. g^{-1} fresh weight) compared to the lowest mean of 25.41 (µg. g^{-1} fresh weight) at condensation zero of $ds.m^{-1}$ of NaCl and 100 mg. $L^{-1}$from arginine.

Table(4): Effect of foliar spraying with arginine on the average content of proline acid (µg.g^{-1} fresh weight) of cowpea plants exposed to salt stress.

Concentrations sodium chloride(ds.m^{-1})	**Arginine concentrations (mg.L^{-1})**			
	0	**50**	**100**	**Sodium Chloride average**
0	30.49	28.44	25.41	28.11
5	31.16	29.71	28.48	29.78
10	42.52	35.09	32.72	36.78
15	46.47	39.91	36.83	41.07
Arginine average	37.66	33.29	30.86	
LSD (0.05)		**Arginine=1.37**	**Sodium chloride=1.**59	**overlap= 2.75**

Table (5) results indicated that there was a significant increase in the average hydrogen peroxide content when treating the plant with NaCl, especially the concentration of 15 $ds.m^{-1}$ Which gave the highest average for the trait 40.17% ($\mu m.g^{-1}$ fresh weight) Compared to control and extreme27.14 ($\mu m.g^{-1}$ fresh weight) With a percentage increase of48.01% of the effect of salinity on the cellular structure of the organelles, causing their oxidation to increase the production of negative free roots, including hydroxyl root, superoxide and hydrogen peroxide, exposing the plant to oxidative damage (Munns., 2002).This is consistent with what were said (2018) Zaki and Mohamed on the bean plant. Also, the treatment with arginine led to an important decrease in the rate of this trait, especially the concentration 100$mg.L^{-1}$ Which gave the lowest average for the trait reached

33.49%($\mu m.g^{-1}$ fresh weight) Compared to the control, which amounted to

40.81 ($\mu m .g^{-1}$ fresh weight), with a percentage decrease of 17.93% for the role of amino acids. In regulating plant growth, oxidation and reduction processes, cellular metabolism, synthesis of growth hormones and maintaining the balance of nutrients in the plant, which reduces the negative effects of salt stress represented by its production of hydrogen peroxide root.(Al-joboori and mohammed.2021;Xia etal.,2014). Also, the interference has a significant effect if it is given concentration15 $ds.m^{-1}$ from NaCl and zero $mg.L^{-1}$ of arginine had the highest mean of 66.53 ($\mu m.g^{-1}$ fresh weight) compared to the lowest average of 18.80 ($\mu m.g^{-1}$ fresh weight) when treated with control.

Table(5): Effect of foliar spraying with arginine on the average content of hydrogen peroxide (μm. g^{-1} fresh weight) of cowpea plants exposed to salt stress..

Concentrations sodium chloride(ds.m^{-1})	**Arginine concentrations (mg.L^{-1})**			
	0	**50**	**100**	**Sodium Chloride average**
0	18.80	37.08	25.54	27.14
5	33.10	33.35	29.92	32.12
10	44.81	40.37	35.33	40.17
15	66.53	50.82	43.17	53.51
Arginine average	40.81	40.40	33.49	
LSD (0.05)		**Arginine=1.41**	**Sodium chloride=1.31**	**overlap= 2.28**

chart (6)outcoms indecated that there was an important raise in the average content of salicylic acid when treating the plant with NaCl, especially the condensation of 15 ds.m^{-1} which gave the highest average for the trait 69.87 (μg.ml^{-1}) compared to the control, which was 64.20 (μg.ml^{-1}). With a percentage increase of 8.33%, the plant's exposure to salt stress activates the defense system inside the plant as a result of the accumulation of Na^{+} and Cl^{-} ions, thus reducing the plant's production of antioxidants other than enzymes, including salicylic acid, which catching free roots and improves the photosynthesis system (Al-Desuquy etal., 2012). This agrees with Al-Saady (2019) on the mung bean. Also, treatment with arginine led to a significant increase in this trait, especially concentration 100 ml. L^{1-} who give the highest average 84.24 (μg.ml^{-1}) compared to control that amounted 60.26 (μg.ml^{-1}) with by a prooprtion raise of 39.79% to provide the appropriate conditions to promote plant growth and activity and preserve its organisms from the effects of the negative roots in the presence of amino acids prepared for the nitrogen and carbon element of the plant (Hassan etal.,2010) .That which increase the ability of the plant to produce antioxidant acids, including nicotinic acid salicylic ,also, the interaction had a significant

effect, as it reached the highest average 95.38 (µg.mL^{-1}) at a concentration of 5 ds.m^{-1} from NaCl and 100 mg.L $^{-1}$ of arginine, while the minimum average was 45.24 (µg ml^{-1}) at a condensation of 5 ds.m^{-1} from NaCl and zero mg. L^{-1} of arginine

Chart (6):effect of foliar spraying with arginine on the average content of salicylic acid (µg. ml^{-1}) Cowpea plant exposed to salt stress.

Concentrations sodium chloride(ds m^{-1})	**Arginine concentrations (mg L^{-1})**			
	0	**50**	**100**	**Sodium Chloride average**
0	63.26	50.35	78.98	64.20
5	45.24	55.55	95.38	65.39
10	67.06	61.30	81.24	69.87
15	67.06	61.30	81.33	75.74
Arginine average	60.26	**61.90**	**84.24**	
LSD (0.05)		**Arginine=1.51**	**Sodium chloride=1.74**	**overlap= 3.01**

Conclusion

When the plant treatment with high concentration of salt lead to significantes increasing in most of physiological properties for plant while arginine has a significantes effect and positive role on the some physiological properties when the plant treated with it also the intraction experiment factors have a significant effect on the most of these properites .

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