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Impact of Amino Acid and Growth Regulator (NAA) on Growth and Chemical Traits of Corn *Zea mays* L. under Salinity Stress

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Abstract: Fields experiments were conducted in the year 2018 in the agriculture and marshes faculty fields - Thiqar university- south of Iraq, to determine the impact of five concentrations of amino acids (0, 1, 2, 3, 4 cm^3. L^{-1}) and four concentrations of NAA (0, 10, 20, 30) ppm on the growth characters (leaves area, dry mass and total soluble sugar/leaves), antioxidant enzymes (ascorbate peroxidase/ leaves (APX), peroxidase/ leaves (POX), guaiacol peroxidase/ leaves (GPX), glutathion reductase/ leaves (GR), and proline/ leaves). The solution of the mix (amino acid and NAA) was sprayed on the corn plant at 30, 45, 60, 75 days after planting date, while the reading was taken at the beginning of the flowering stage. The corn plants were treated with amino acid 1.5 ml. L^{-1} x 30 ppm NAA gave the highest total carbohydrate/leaves, protein/leaves and the lowest enzyme activities of corn plants, whereas the treatment 0 cm^3. L^{-1} x 0 ppm NAA gave the highest values of proline/leaves, guaiacol peroxidase/leaves, glutation reductase/leaves and the lowest means of total carbohydrate/ leaves and protein/leaves indicating that the mix solution (amino acid and NAA), might be enhanced the growth of the corn plant and protect plant cells from the effects of salinity stress.

***Keywords*:** Amino acid, NAA, Growth, Corn, *Zea mays*, Salinity stress

Corn is essential for global food security, Maize (*Zea may* L.) is an important food and feed crop which ranks third after wheat and rice in the world. The growth regulators dramatically alters the growth and is often a crucial factor in secondary product accumulation. There are several reasons of low growth of plants, among cultivated plants under the salinity stress consequently there is reduction of plant growth, yield and chemical characters of corn seeds. Hence there is a need to increase the ability of the corn plant to tolerate the salinity for higher maize production of better quality. This study was conducted to examine the effect of amino acids and NAA on the production of total carbohydrates, protein contents and the activity of antioxidant enzymes of corn plants cultivated under the salinity stress.

MATERIAL AND METHODS

Preparation of soil and the experimental factors: The experiment was conducted into autumn season 2019 at Thiqar university in south of Iraq to determine the impact of amino acids (0, 1, 2, 3, 4 cm^3. L^{-1}) and naphthalene acetic acid NAA (0, 10, 20, 30 ppm) on the growth traits, (leaves area, dry mass and total soluble sugar/ leaves), antioxidant enzymes (Ascorbate peroxidase/ leaves (APX), peroxidase/ leaves (POX), guaiacol peroxidase/ leaves (GPX), glutathion reductase/ leaves (GR), and proline/ leaves. The solution of the mix (amino acid and NAA) was sprayed on the corn plant at 30, 45, 60, 75 days after planting date and data was collected at the beginning of the flowering stage. The corn crop was cultivated under salinity of 8.14 milimos.cm^{-1}. The amino acids solution used in this study was contained aspartic acid 2.5 % thrionine 0.45%, serine 0.56%, glutamic acid 0%, glycine 0.5 %, alanine 10.0 %, proline 0.38% , valine 0.68 %, cystine 0.44%, methionine 0.18%, isoleucine 0.52%, tyrosine 0.38%, phenylalanine 0.32 %, histidine 0.12 %, lysine 0.4 , arginine 0.2 % and tryptophan 0.2%).

The mixed solution was sprinkled in the early morning on the vegetative part of the corn plant until the wetness and the fall the first drop of the solution from the plant. The plots were plowed two times orthogonal and then divided into 3 replicates, each replicate has 20 experimental unite with an area of 2 m^2. The soil physico - chemical properties are presented in Table 1. Super Phosphate triple P_2O_5 was added at 132 kg/ha as a single batch when preparing the field. Urea fertilizer 46% N was added by 150 kg/h in two equal installments, the first at the time of sowing and the second when the corn plant high became 30 cm. The experiment was on Baghdad 3 variety was sown on Augst 15, 2019.

Preparation of solutions: A substance added to the solution (liquid soap) to reduce surface tension and to ensure complete wetness.

Experimental traits: The growth traits (a leaf area, dry mass and total soluble sugar/ leaves), enzyme activities (APX, GPX, GR and POX, and proline/ leaves) were estimated

at10% flowering stage. Total soluble sugar was estimated according to (Dey 1990). The nitrogen content was measured by the standard Kjeldahl procedure (MS1194, 1991). One g of the dry leaves sample was put into a digestion tube and the reaction was catalysed with the addition of 12 ml sulphuric acid. The digestion tubes were shaken vigorously and after 45 min the digestion tube rack was removed and cooled for 20 min. About 80 cm^3 distilled water was added to each of the digestion tubes with 25 ml boric acid plus indicator in conical flasks. The flasks were placed into a distillation unit (2100 Kjeltec distillation unit, FOSS tecator) and finally titration was done using 0.1000 N HCL.

Enzyme extraction and assay: About (0.5g) of the corn leaf sample was homogenized in an ice-cold 0.1 M phosphate buffer (pH=7.5), the protein content of the samples was determined using a Bovine albumin method (Bradford 1976).

Ascorbate peroxidase (APX): Ascorbate peroxidase was assayed spectrophotometer at 290 nm in an UV (model M 36, Beckman,CA, USA) equipment.

Peroxidase (POX): Peroxidase activity was assayed as an increase in optical density due to the formation of tetra-guaiacol (Castillo et al 1984).

Guaiacol peroxidase (GPX): GPX activity was determined according to Upadhyaya et al (1985).

Glutathione reductase (GR): Glutathione activity was assayed according to (Siram et al 2002).

Proline estimation: Approximately 0.5 g of fresh leaf material was homogenized in 10 cm^3 of 3% aqueous sulfosalicylic acid, left for 3hrs for extraction to complete centrifuge at 1500 g for l0 min, 2 ml of supernatant was added to 2ml glacial acetic acid and 2 ml acidic ninhydrin, boiled at 100 ° C in a water bath for 60 min, and then stop the reaction by placing in an ice path. Then 4 ml of toluene was added and mix vigorously, then observed spectrophotometrically at 520 nm, using toluene as blank. At 4^0C, the reagent is stable for 24 ha and standard curve was used with concentration from 0 - 512 µL (20-100 µg/ml) of L-proline (Bates et al (1973).

Statistical analysis: All samples were processed in triplicate and their means were calculated. The statistical analysis was performed using the software SPSS (version 20).

RESULTS AND DISCUSSION

All the traits were influenced by using different levels of amino acids and NAA concentrations with significant differences in the growth trait (leaves area, dried matter and total soluble sugar (Fig. 2, 3, 4), where the treatments (3 cm^3. L^{-1} amino acids X 20 ppm NAA have the highest means of 4731.48 cm^2, 143.22 gm, 211.34 µg/g FW, respectively, while the control (0 cm^3. L^{-1} amino acids X 0 ppm NAA) gave the lowest means (2772.35 cm^2, 57.31 gm, 80.44 µg/g FW respectively). The correlation analysis shows a significant positive correlation (p<0.01) between the amino acids and NAA concentrations with the growth characters (leaves area, dried matter and total soluble sugar content in leaves of the corn plants) (r= 0.81**, r= 0.83**; r= 0.80** respectively). This indicated that the corn plants treated with 3 cm^3. L^{-1} amino acids X 20 ppm NAA enhanced the ability of the corn plants to prevent the adverse effects of salt with highest levels of the growth parameter, and resisted the rising salts to the plant body.

Earlier worker reported that amino acids are important components working to reduce of free radicals risks, where the plant can use the amino acids in different signaling pathways according to their stage of development (Foolad 2007, Gill and Tuteja 2010, Rennenberg and Herschbach 2014). The current study indicated that the growth status of the plant improved when amino acids sprayed on leaves indicating that the amino acids helped protect the plants from free radicals and osmotic protection. This is consistent with the findings of Demiral and Turkan (2006), Hamdia and Shaddad (2010). In this study the increasing of growth parameter in corn plants may be due to the role played by the amino acids used in increasing the production of some plant hormones such as tryptophan, which is the main substance in the process of biosynthesis of auxine 3-Indol acetic acid, also the phenylalanine and ornithine are involved in process of the vital construction of gibberellins. This increase in plant hormones has increased the frequency of cell division, elongation and size, leading to increased plant growth indicators such as leafy area and dry weight as well as an increase in total soluble sugars content.

In this study, increasing the concentration of amino acids and NAA lead to reduce the average of enzyme activities (APX, POX, GPX, GR) and proline content in leaves of corn plants to a certain limit. The treatment 3 cm^3. L^{-1} amino acids X 20 ppm NAA has given the lowest activities in those antioxidant enzymes and proline (3.98, 4.82, 41.37, 26.22 m.mol. min^{-1}. mg^{-1} protein and 5.58 µg/g FW, respectively as compared with 0 cm3. L^{-1} amino acids X 0 ppm NAA which

Table 1. Physico-chemical properties of soil at the experimental site

Physico-chemical properties of soil	pH	O.M (%)	E.C (milimos. cm^{-1})	C.E.C (meq/100 g soil)	N (%)	$Po_{4\,(\%)}$	Soil texture
	7.**3**	1.43	9.23	1.27	0.87	0.03	Silty loam

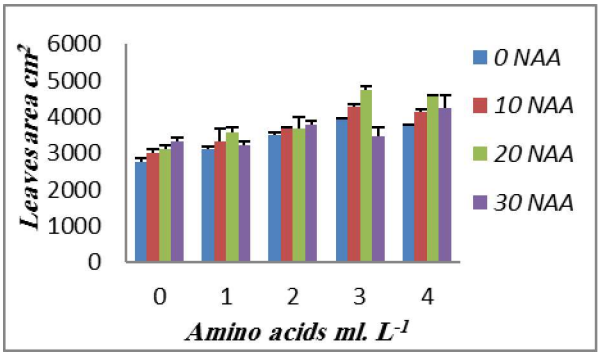

Fig. 1. Leaves area (cm^2)

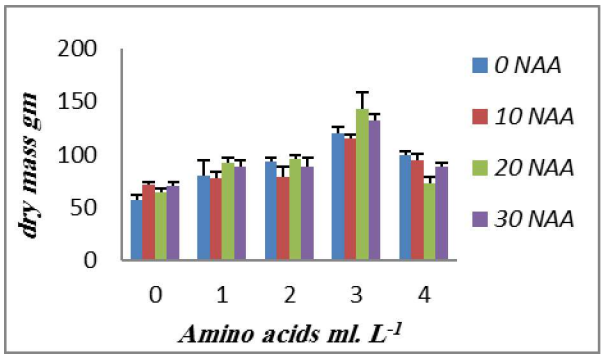

Fig. 2. Dried matter (gm)

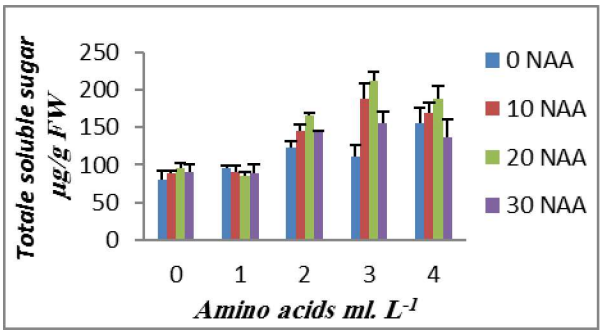

Fig. 3. Total soluble sugar

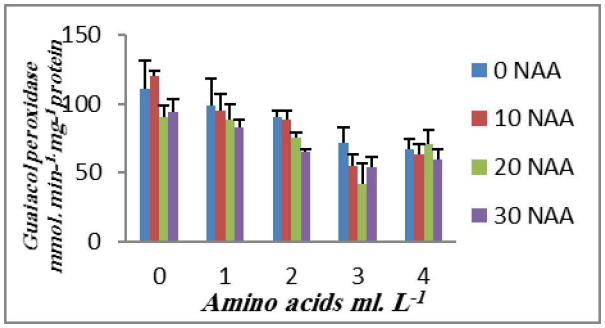

Fig. 4. Guaiacol peroxidase/leaves

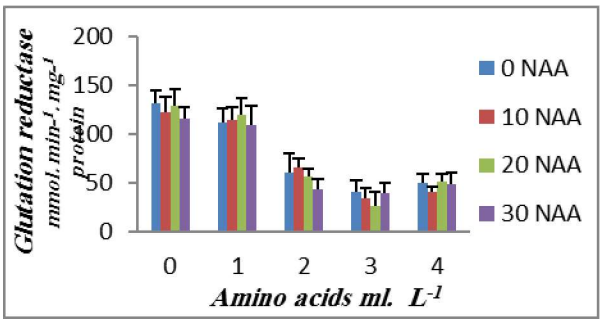

Fig. 5. Glutation reductase/leaves

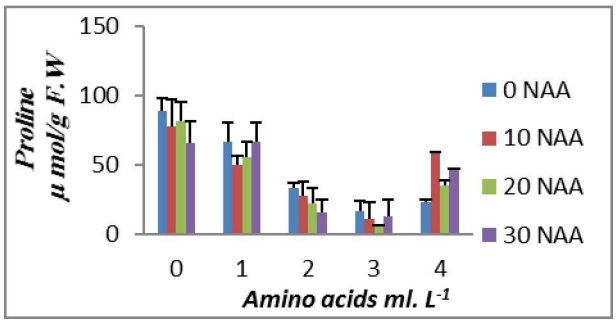

Fig. 6. Proline/leaves

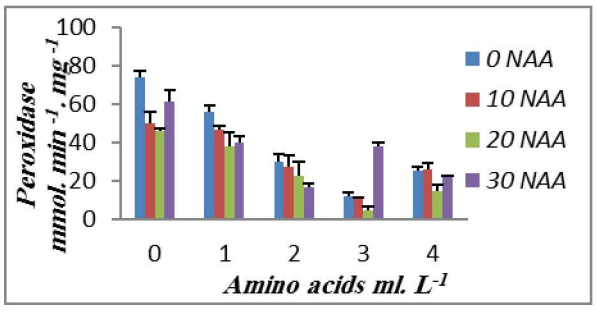

Fig. 7. Ascorbate peroxidase/leaves

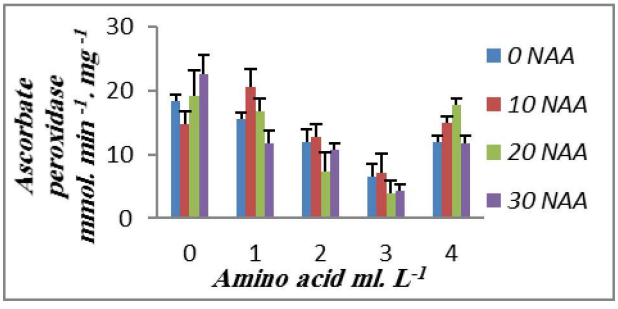

Fig. 8. Peroxidase/leaves

have given the highest activities of all of these enzymes affected by the salinity of the soil (74.30, 120.32, 131.33 m.mol. min $^{-1}$. mg^{-1} protein and 88.37 µg/g FW) for POX, GPX, GR and proline content, respectively, whereas the highest activity of APX observed at 0 cm^3. L^{-1} amino acids X 30 ppm NAA 22.86 m.mol. min $^{-1}$. mg^{-1} protein.

Correlation analysis shows that there is a significant negative correlation (p<0.01) between the amino acids and NAA concentrations with enzyme activities (APX, POX, GPX, GR) and proline content (r=-0.71, r= -0.77; r= -0.81 r= -0.70 r= -0.79 respectively),. This result indicates that using 1.5 ml. L^{-1} amino acids X 20 ppm NAA protect the maize plant cells from the harmful effects of salts.

In the current study, the salinity induced a marked effect on the antioxidant activities (0 cm3. L-1 amino acids X 0 ppm NAA), an increase in the enzyme activities of (APX, POX, GPX, GR) and proline content at this level could be indicative of an increased production of ROS and reduce the oxidative damage (Chaparzadeh et al 2004). The antioxidant enzymes such as APX, POX, GPX, GR) and proline content activities increased in the treatment (0 cm^3. L^{-1} amino acids X 0 ppm NAA), thereafter, the activities of all the antioxidant enzymes increased, indicating that's the solution of amino acids and NAA might be assisting neutralize the harmful effect of salts in the soil solution. Anioxidant enzymes viz.,(APX, POX, GPX and GR) are the major enzyme responsible for H_2O_2 scavenging during stresses, however, in this study all these H_2O_2 scavenging enzymes showed uniform increase when dont used the amino acids and NAA solution there after, declined when the increasing in the solution concentrations applied, suggesting that all these enzymes play important role in H_2O_2 scavenging at salinity stress and the amino acids and NAA application was enhanced the plant cells ability to resistans the salt stress when they incresesd in their concentration in the plant cells. The decline in antioxidant enzymes (APX, GPX, GR, POX) and proline in the crrent study when treatment with amino acids and NAA (3 cm^3. L^{-1} amino acids X 20 ppm NAA)might be accompanied by accumulation of other organic solutes (saccharides, protein and total amino acids) to some extent, lead tolerance form plants to salt stress through osmoregulation, that using the organic solutes rather than these enzymes and proline.

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CONCLUSION

This study revealed a significant negative effects occurred by the salinity of soil to the growth parameter of corn plants, and the amino acids and NAA solution has a significant role in reducing the harmful effects of salts and enhance the ability of plants to grow in the salinity soil.

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