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Enhancement in Okra (*Abelmoschus esculentus*) Growth Performance Under Salt Stress Using Exogeneous Application of L- tryptophan

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ABSTRACT

Salinity is one of the major stress factors that has a substantial impact on agricultural resilience and concerns global food safety. L-tryptophan being an essential regulator with specialized activities in plant functioning, as well as an increase in tolerance to various abiotic stressors. A hydroponic experiment was carried out to determine the effective role of L-tryptophan in okra seedlings in saline environment in terms of growth, physiological, ionic, and antioxidant properties. Salinity was imposed in growth medium with two levels of NaCl (Control and 80 mM NaCl) whereas one level of L-tryptophan (50 µM L^{-1}) administered externally in both combined and single forms. MDA and H_2O_2 contents increase while plant dry matter, chlorophyll content, relative water contents, membrane stability index, K^+/Na^+ ratio decreases due to salt stress. Salt toxicity was reduced when L-tryptophan was added as illustrated by increased relative water contents, membrane stability index, K^+/Na^+ ratio, as well as suppression of MDA and H_2O_2 generation. Our findings suggested that L-tryptophan increased salinity tolerance in okra, which could be a cause of viable sustainable production from salt-affected soils.

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INTRODUCTION

Soil salinization is the most severe abiotic factor threatening agricultural yields worldwide, especially in precipitation scarce regions (Moreira *et al*. 2020). Salt accumulation is promptly spreading, resulting in a damage of 10 mha of land per year and a 50% damage of cultivable land by 2050 (Ali *et al*., 2021) When plants are exposed to saltwater, their water absorption rate decreases significantly. This has an effect on inter- and intracellular water levels, inhibiting cell expansion and decreasing stomatal activity. High salt concentrations in growing media have a negative impact on plant biomass, physiology, mineral ion buildup (El Emin *et al*., 2020), PSII reaction destruction (Akbar *et al*., 2021), and biochemical damage due to ROS formation (Xu, *et al* 2018).

To develop salinity tolerance, various exogenous chemicals have been used to mitigate the harmful effects of salt stress. L-tryptophan functions as a cofactor in the activity of enzymes (DNA and RNA) as well as hormones (Hanci and Tunser, 2020). L-tryptophan protects a diversity of vegetation against abiotic stress similar as salt stress (Mushtaq *et al*., 2021), drought (Danish *et al*., 2020), heavy metals (A El-Shanhorey and Ahmed, 2020), and heat stress by stimulating roots, upregulating

antioxidant enzymes, transforming appearance of numerous salt tolerant genetic factors, upgraded plant water associations, and actual foraging of reactive oxygen species (RO (Cheng *et al*., 2020).

Okra is a nutritious vegetable crop that is widely grown. Although okra cultivation has increased in Pakistan in recent years, productivity has been limited in many areas due to soil and water salinization (Qureshi, 2020). Pakistan has an arid to semi-arid climate, salt stress is a major issue for okra production. The main objective of the current experiment is to look into the acclimating role of L-tryptophan on plant growth, physiological and biochemical properties, and ion homeostasis in okra seedlings exposed to salt stress.

MATERIALS AND METHODS

H A rain-protected wire house was used for the solution culture experiment, and sterilized seeds of the okra variety Anmol were sown in moist sand culture. Nursery was raised in sand shifted at the two-leaf stage onto a styro-foam sheet moving on water in plastic tubs having 50 L capacity. The necessary salt levels (control and 80 mM) were created by combining the preferred amount of NaCl with L-tryptophan at a concentration of 50 µM. The experiment was carried out in a completely randomized design arranged in split plot arrangements, with three replications. Treatments comprised of control (T_1), Salt stress 80 mM NaCl (T_2), 50 µM L-tryptophan (T_3), 80 mM NaCl + 50 µM L-tryptophan (T_4). Hoagland solution at half strength (Hoagland and Arnon, 1950) was used as a nutrient media and pH of the solution maintained at 6.0 $\pm$ 0.5.

Plant growth, leaf area and chlorophyll determination

After 28 days of stress, plants were harvested. Root and shoot fresh and dry weight were recorded with analytical balance, while root and shoot length were recorded with the help of measurement scale. Chlorophyll contents was measured by using SPAD meter.

Relative Water Contents (RWC)

Leaf samples were freshly sampled, and their fresh weight was recorded soaking in water for 24 hours, after which turgid weight (TW) of the samples were recorded. The samples were oven dried at 80°C recorded as dry weight (DW) of the samples. Leaf relative water contents were determined by putting the values in the following equation (1999, Lazcano-Ferrat and Lovatt).

RWC % = (FW-DW) / (TW-DW) × 100

Membrane Stability Index (MSI)

Leaf strip (100 mg) were heated in deionized water (10 ml) in a water bath for half an hour at 40 °C, and electrical conductivity (EC) of the solution was recorded (C_1). Same samples were heated at 100 °C for 10 minutes after which EC was recorded as C_2 (Sairam and Saxena, 2000). Membrane stability index was determined using the following equation.

MSI % = [1- (C_1/C_2) × 100]

K^+ and Na^+ concentration

A plant sample (0.5 g) was extracted and processed using a flame photometer following the procedure explained by Ali *et al*. 2021 for the Na^+ and K^+ determination.

Antioxidant stress indicators

The contents of lipid peroxidation (MDA) were determined by determining the absorbance of solution at 532 nm, as described by Jambunathan, 2010. The amount of hydrogen peroxide (H_2O_2) generated in the samples was measured using the Masoumi *et al*. 2000 methodology, which involved using a spectrophotometer to calculate absorbance at 390 nm.

Statistical analysis

The data were investigated statistically using the statistical tool Statistics 8.1. The bars in the graph represent the average of three replicates, while the error bars represent the standard deviation. The average values were compared using the LSD test at a probability of 5% to compute and estimate the basis of deviation (Steel *et al*. 2007).

RESULTS

P L-tryptophan increase fresh and dry biomass of okra seedlings under salinity stress

Analyzed data in Table 1 exhibit effect of exogenously applied L-tryptophan on fresh and dry biomass, shoot and root length as well as leaf area of okra seedlings during NaCl stress. In comparison to control, it was observed that application of NaCl in the growth interface cause noteworthy reduction in plant growth attributes and reduction in shoot and root length (70 % and 60 %), fresh (62 % and 53 %) and dry weight (68% and 57%) along with leaf area (%) was observed under elevated salt stress (80 mM NaCl). Sole application of L-tryptophan depicts no significant improvement in okra plant growth attributes however, L-tryptophan supplementation @ 50 µM remarkably enhanced plant height, root and shoot fresh and dry contents of okra seedlings when applied with combination of salt stress.

Table 1. Interactive effect of Salinity and L-tryptophan application on fresh and dry biomass of okra seedlings. Values depict the mean of three replicates and values not sharing the same letter in each column differ significantly according to the LSD test (P <0.05) level.

Treatments	Root Length (cm)	Root Fresh Weight (g)	Root Dry Weight (g)	Shoot Length (cm)	Shoot Fresh Weight (g)	Shoot Dry Weight (g)
Control	10 a	4.9 a	1.01 a	21.8 a	12 a	3.2 a
80 Mm NaCl	4.8 c	2.25 b	0.58 c	14.3 b	6.7 b	1.41 c
50 µM L- tryptophan	10.3 a	5.0 a	1.03 a	20 ab	12.3 a	3.27 a
80 mM NaCl + 50 µM L-tryptophan	6.9 b	3.1 b	0.78 b	17.9 ab	8.1 b	1.94 b

L-tryptophan improved okra plant physiology under salt stress

Exalted salt level proved hazardous in relations to membrane stability index (MSI), relative water contents (RWC), and chlorophyll contents (Figure 1). Maximum relative water contents and membrane stability index were noted in control where no NaCl stress was applied. After subjected to salinity stress, L-tryptophan application partially recovered plant water associations and membrane stability and this retrieval is more protruding when L-tryptophan was applied in association with the salt stress. Results also discovered the decrease in chlorophyll contents with increasing concentration of salts in the growth medium as compared to control, maximum reduction (60 %) was noted at treatment with high salt level (80 mM). Provision of L-tryptophan mitigates the negative impacts of salt stress on chlorophyll contents and show improved chlorophyll values as depicted in the current project.

L-tryptophan improved K^+/Na^+ ratio in okra seedlings under salt stress

Interaction of zinc and salt stress on the potassium to sodium (K^+/Na^+) ratio in okra seedlings are depicted in Figure 2. In comparison to the control, the improved salt strength in the growth channel resulted in a significant rise in the amount of Na^+ and a significant drop in potassium concentration in examined shoots of okra seedlings. The propensity was contradictory in situation of K^+ uptake by showing smallest K^+ amount at extreme salt quantity (80 mM) in the current experiment, improvement in K^+ values were observed when L-tryptophan was applied with salt stressed condition in okra. The excess uptake of sodium and decreased potassium concentration by enhancing salt stress concluded in lower K^+/Na^+ relationship. L-tryptophan application under salinity stress alleviated the antagonistic responses of salinity and maximized the K^+ absorption in okra seedlings.

L-tryptophan suppress the production of MDA and H_2O_2 contents

In the current study, an extreme amount of Na^+ ions (80 mM NaCl) in the growing interface amplified the malondialdehyde (MDA) contents and hydrogen peroxide (H_2O_2) in okra seedlings significantly (P < 0.05). The results also showed that applying L-tryptophan reduced the levels of MDA and H_2O_2 in okra seedlings. When exposed to salt stress, MDA and H_2O_2 levels in okra leaves increased by and percent when compared to controls. However, when matched to foliage grown solely in a salty medium, the use of L-tryptophan significantly mitigates the hazardous effect of salinity and suppresses the MDA and H_2O_2 contents by up to and percent as presented in Figure 3.

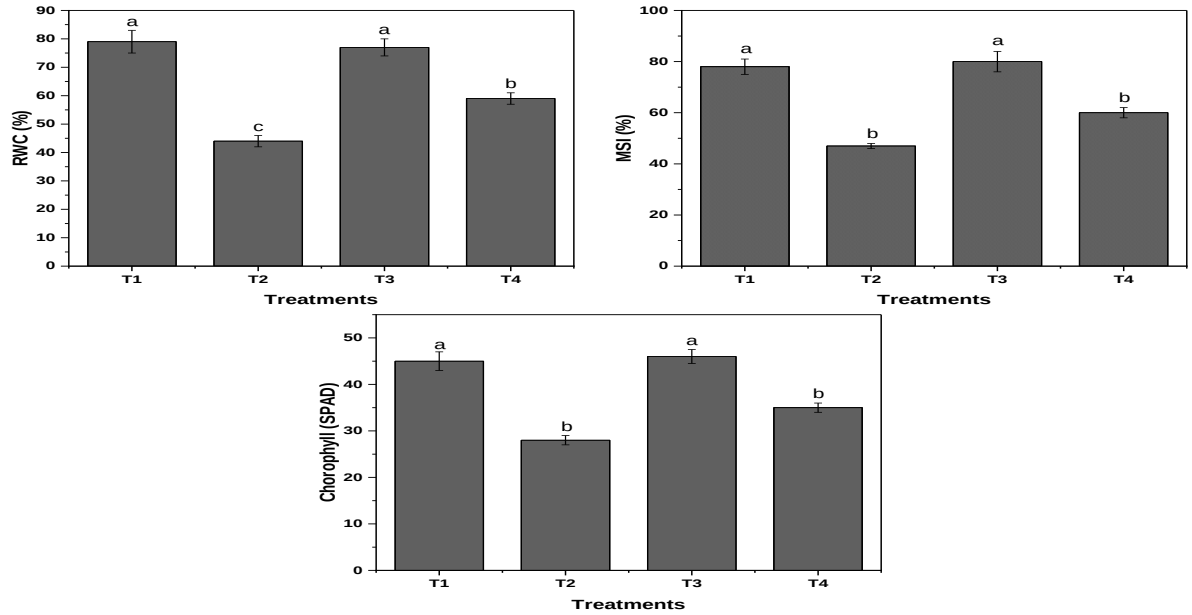

Figure 1. Interactive effect of Salinity and L-tryptophan application on physiological attributes of okra seedlings. Values depict the mean of three replicates and bars not sharing the same letter differ significantly according to the LSD test (P <0.05) level. Treatments comprised of Control (T_1), Salt stress 80 mM NaCl (T_2), 50 µM L-tryptophan (T_3), 80 mM NaCl + 50 µM L-tryptophan (T_4).

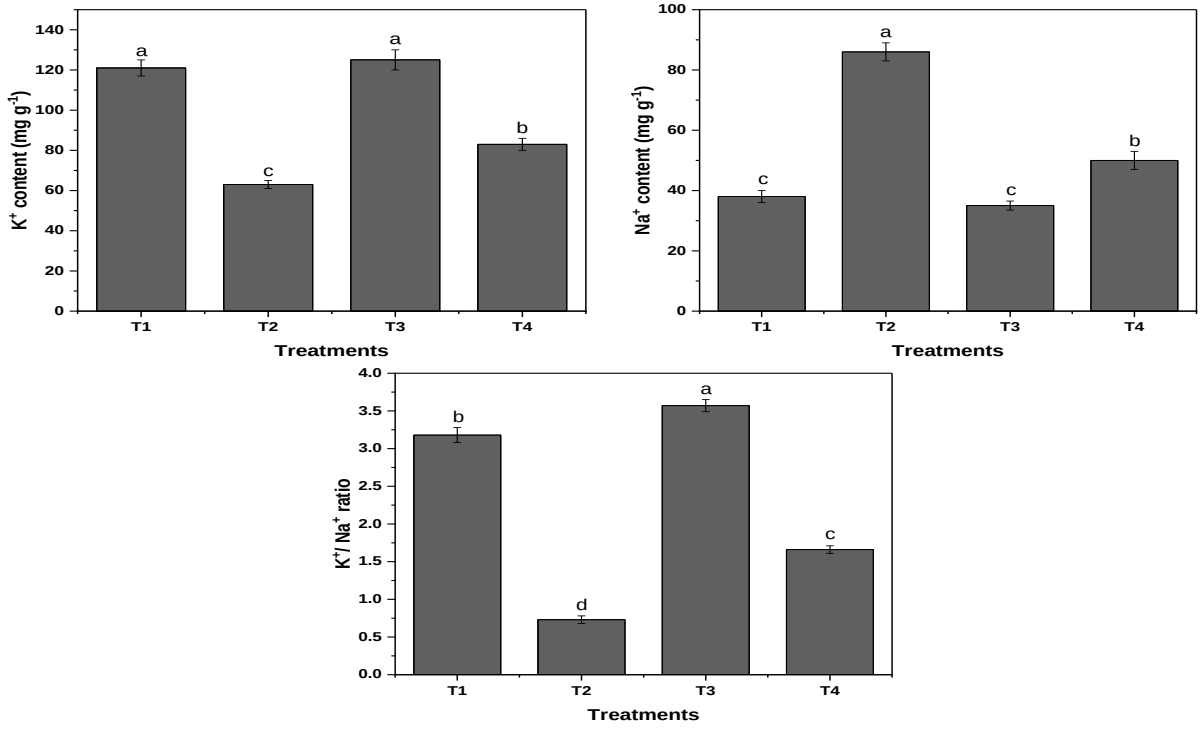

Figure 2. Interactive effect of Salinity and L-tryptophan application on K^+/Na^+ of okra seedlings. Values depict the mean of three replicates and bars not sharing the same letter differ significantly according to the LSD test (P <0.05) level. Treatments comprised of Control (T_1), Salt stress 80 mM NaCl (T_2), 50 µM L-tryptophan (T_3), 80 mM NaCl + 50 µM L-tryptophan (T_4).

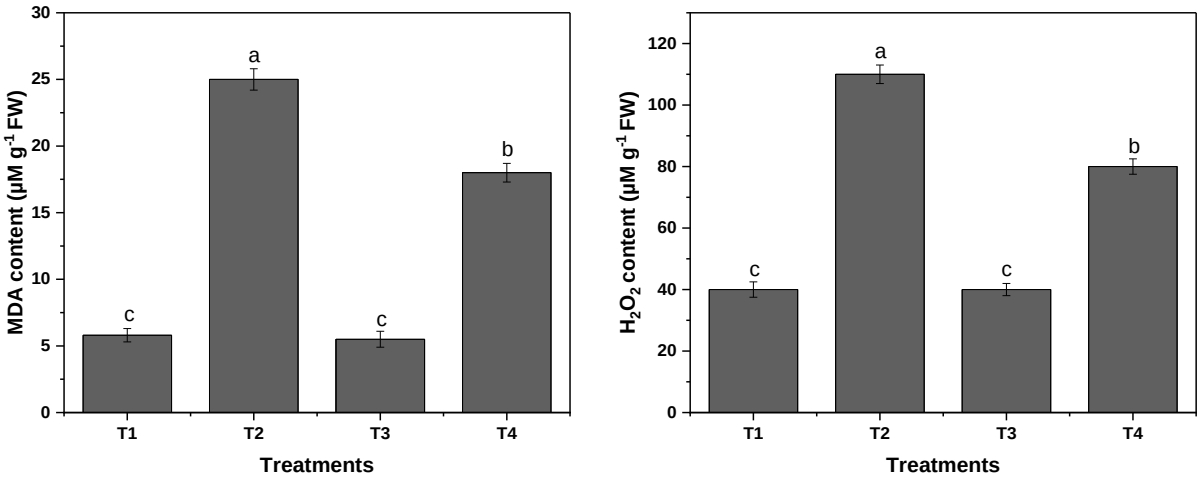

Figure 3. Interactive effect of Salinity and L-tryptophan application on MDA and H_2O_2 contents of okra seedlings. Values depict the mean of three replicates and bars not sharing the same letter differ significantly according to the LSD test (P <0.05) level. Treatments comprised of Control (T_1), Salt stress 80 mM NaCl (T_2), 50 µM L-tryptophan (T_3), 80 mM NaCl + 50 µM L-tryptophan (T_4).

DISCUSSION

Elevated salinity in the growth channel results in degeneration of plant biomass, physiological attributes, biochemical traits as well as ionic contents that eventually disturbs the crop production while L-tryptophan considerably amends the lethal effect of salinity. Reduction in growth attributes under salinity is attributed to low nutrient absorption, disturbed photosynthesis and plant physiology along with ROS production (Kumar *et al*., 2020). High buildup of Na^+ ions in plants extremely disturbs plant gas exchange traits and impaired production of life sustaining components. Provision of L-tryptophan to salt stressed plants decreased Na^+ inflow in disturbed roots, enhanced leaf area, plant height and photosynthetic action alongside with antioxidant enzymes activities (Yasmeen *et al*., 2020).

Excess Na^+ disturbs each feature of plant functioning by interfering with stomatal regulation, photosynthesis ability and transpiration (Aliniaeifard *et al*., 2016). The major cause of decrease in RWC under salt stress may be plant ionic difference, which reduces the availability of water in the growth medium (Abbasi *et al*., 2016). With increasing NaCl concentration, there was a decrease in membrane stability index and chlorophyll contents, which depreciates membrane stability and diminishes leaf tenderness as well as ROS accumulation in membrane at different cells, particularly in salt sensitive species of plants (Kumari *et al*., 2018). Salt stress significantly reduced overall growth and leaf gas exchange of okra seedlings by causing Na^+ toxicity, and osmotic stress (as evident by reduced RWC under salt stress) (Figure 1). Both these abiotic environmental stresses under saline condition further induce ROS production in leaves. The oxidative injury is the major importance of ecological stresses owing to disproportionate buildup of ROS. As the harshness of oxidative impairment to the cell membrane by H_2O_2 is commonly measured as MDA contents (Haroon *et al*., 2021). In current study we found that untreated okra seedlings showed 2 to3 times higher H_2O_2 (ROS) and MDA contents under salt stress compared with control. Such higher production of ROS leads to reduction in membrane stability, thus showed lower MSI okra plants under salt stress. Besides oxidative stress, reduction in RWC due to lower water uptake is the direct result of osmotic stress component of salt stress and reduction in RWC leads to poor plant biomass (Ali *et al*., 2021). Salt stress reduced RWC in okra seedlings which was positively correlated with MSI, indicating salt stress disrupted membrane permeability by reducing RWC and increasing ROS production (Behzadi *et al*., 2021). L-tryptophan effectively alleviates the restraining action of salinity with refining root/shoot ratio and modifying the improved water absorption by plants (Weisany *et al*., 2014). The free ROS production can be balanced by using L-tryptophan, which increases the antioxidant reaction of

plants at the cellular level by activating definite antioxidant enzymes which increase membrane stability (Tavallali *et al*., 2022).

As a result of salt stress, additional Na^+ assembling occurs within plant cells. Na^+ can enter cells due to the presence of Na^+ transport channels in cell membranes (Ali *et al*., 2016). As the amount of NaCl applied increased, the amount of Na^+ in the growth medium increased dramatically, resulting in low plant biomass. Lower K^+ uptake in a saline environment may be due to the reasonable absorption of Na^+ and Cl^- with specific food supplements such as Ca^{2+}, K^+ and Mn^{2+}(Shabala and Pottosin, 2014). Mustafa *et al*. (2018) discovered similar results in maize. L-tryptophan supplementation decreased Na^+ uptake by roots, resulting in increased K^+ increase, osmotic alteration, and an enhanced K^+/Na^+ ratio (Morshedi and Farahbakhsh, 2012). The application of L-tryptophan in the current study allows the okra plant to maintain higher K^+ levels, which is consistent with the findings of Sharma *et al* 2018. In current study, we found that salt stress significantly reduced K^+ uptake and increased Na^+ uptake in in root and shoot of okra seedlings, while L-tryptophan application considerably reverted the response to these traits to salt stress. One of the L-tryptophan induced ameliorative effects was the maintenance or retention of higher K^+ in the root and shoot under salt stress. Upon salinity stress, K^+ deficiency may exit due to increase in the uptake of cytosolic Na^+ and higher production of ROS either by higher Na^+ uptake or disruption of Photosynthetic process, thus generates several radical oxygen species and reduces plants growth. Thus, maintenance of higher K^+ is crucial for plant survival under saline conditions. Here we found that, L-tryptophan application regulates and maintained K^+ homeostasis by regulating the transcript of several K^+ transporters.

As evidenced by higher oxidative stress indicators (MDA and H_2O_2) during salinity, salt-encouraged oxidative impairment may be liable for plant membrane rupture, electrolyte escape, fat peroxidation, and nutrient uptake hang-up (Mukarram *et al*. 2021). These findings are consistent to the findings of Manai *et al*. (2014), who determined that oxidative damage is increased in tomato seedlings under salinity. A substantial decrease in MDA and H_2O_2 after L-tryptophan use, on the other hand, could be the result of decreased Na^+ absorption, enriched plasma membrane strength, and low contact of tomato roots to a salty environment.

CONCLUSION

Salinity toxicity alters morpho-physiological characteristics as well as photosynthetic and biochemical properties in okra seedlings, resulting in significant yield and economic losses. L-tryptophan application improved plant biomass, photosynthetic and antioxidant capacity, as well as K+/Na+ ratio in okra plants subjected to NaCl stress. Our findings can help researchers working on vegetable plants understand the role of L-tryptophan in mitigating abiotic stress, including salinity, and open up new avenues for its application in agriculture.

CONFLICT OF INTEREST

The authors declares that they have no conflict of interest.

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