Amelioration of Defense System by Exogenous Citric Acid Application to Lettuce (*Lactuca sativa* L.) Plant

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ABSTRACT

Improper agricultural practices over the decades symbolize a serious problem for various crops productivity. Citric acid (CA) ($C_6H_8O_7$) has demonstrated a superior role in abiotic stress resistance in plants. The current investigation was implemented to test the effectiveness of citric acid root application before and after transplanting. The treatments were: T1 = root soaking (RS) 0.5 g/L, T2 = (RS) 0.75 g/L, T3 = (RS) 1 g/L, T4 = soil application (SA) 0.5 g/L, T5 = (SA) 0.75 g/L, T6 = (SA) 1 g/L, T7 = (RS) 0.5 g/L + (SA) 0.5 g/L, T8 = (RS) 0.75 g/L + (SA) 0.75 g/L, T9 = (RS) 1 g/L + (SA) 1 g/L, and T10 = untreated (control). Overall, the findings demonstrated that the administration of CA in both seasons had a considerable impact on all vegetative development. With the exception of T9, all treatments enhanced growth parameters when compared to the untreated treatment; T7 showed the most remarkable improvement when compared to all other treatments. Citric acid has the potential to decline the harmful effects of abiotic stress in lettuce by inhibiting uptake of Na^+ and Cl^- and by stimulating the antioxidant enzymes such as SOD, POD, CAT, PAL, and PPO.

Keywords: Citric acid - Lettuce - Antioxidant enzymes - Root soaking - Soil application

INTRODUCTION

Secondary salinization is the most common global problem restraining agricultural production (Zhu, 2001). Abiotic stress causes a potential osmotic stress, ion toxicity, and the creation of the reactive oxygen species (ROS) (Shu et al., 2014). The imbalance of ROS leads to programmed cell death and oxidative damage under salinity conditions (Roncarati et al., 2008). Secondary salinization extremely increases, particularly with high electrical conductivity in soil, which may occur when there is a long period of intensive farming, irregular irrigation, inadequate drainage, and inappropriate fertilizer application (George et al., 1997, Ozsan et al., 1983 and Wang et al., 2003). Unsuitable electrical conductivity (EC) and excessive levels of soluble salts result in diminished availability of minerals that are necessary for crop growth and development (Yadav et al., 2011). To reduce the negative effects of salt stress and help plants to develop a resistance to it, a variety of agronomic and physiological techniques are used. While plants increase salinity tolerance by mitigating ion compartmentation, osmotic adjustment, and the stimulation of antioxidant enzymes (Ahmad Hussein et al., 2018 and Mittler, 2002).

One of the most widely consumed salad vegetables, especially in arid regions like Egypt, is lettuce. Pink and Keane (1993) reported that the lettuce is the most common vegetable species that grows normally in a cool temperature and belongs to the compositae family (*Lactuca sativa* L.). Lettuce is a good source of folate, fibers, iron, and vitamins and has several nutritional advantages, such as reduced fat, calorie, nitrate, and sodium content (Ncama and Sithole, 2022). On the other hand, it hydrates and gives the body a sense of fullness. Crisphead lettuce is widely used in the making of fast food and low-energy meals. According to (Iammarino et al., 2014), it is a popular leafy green vegetable crop that contains nitrate NO_3^- in consumed leaves.

Nitrate by itself is relatively non-toxic, while high nitrate levels are dangerous to human especially infants, because they have a lower level of nitrate reductase (Breś et al., 2022). Lettuce is considered to be a relatively salt-sensitive vegetable; salinity can cause a high concentration of NO_3^- in the fresh green mass. The dramatic accumulation of NO_3^- in vegetable crops related to secondary salinization was studied (Dalio et al., 2013 and Debouba et al., 2007).

One approach to enhance vegetable production, like lettuce, under salinity conditions focused in recent years is organic acids (Ncama and Sithole 2022). Citric acid (CA) is one of the most prevalent organic acids, which modifies soil physical properties (EC) and forms complexes with elements to increase their availability (Campbell, 2010). Plant roots exude citric acid naturally, which enhances the plant's resistance to different abiotic and biotic stress and thus increases yield (Pérez-Labrada et al., 2016). The application of CA affects the secondary metabolites and enhances plant production growing in stressed soil by modifying antioxidant enzymes (Ali et al., 2024, Mallhi et al., 2020 and Tahjib-Ul-Arif et al., 2021). One of citric acid's main roles is to reduce plant phytotoxicity, which improves total biomass and quality (Najeeb et al., 2009). To our knowledge, no information on the citric acid application methods for improving lettuce plants has been published. Hence, the current research was aimed to assess the effect of citric acid root soaking and root application on healthy lettuce growth and quality under probable secondary salinization.

MATERIALS AND METHODS

Experimental design and growth conditions:

A field experiment was performed in a randomized complete block design (RCBD) at a private farm in Giza Governorate, Egypt, during two consecutive seasons (2022 and 2023). The experiment was comprised of ten treatments with three replicates to test the effect of citric acid on lettuce performance *(cv Big bell)*. The treatments were: T1 = root soaking 0.5 g/L, T2 = root soaking 0.75 g/L, T3 = root soaking 1 g/L, T4 = soil application 0.5 g/L, T5 = soil application 0.75 g/L, T6 = soil application 1 g/L, T7 = root soaking 0.5 g/L + soil application 0.5 g/L, T8 = root soaking 0.75 g/L + soil application 0.75 g/L, T9 = root soaking 1 g/L + soil application 1 g/L, and T10 = untreated (control). Seedlings of lettuce were selected homogeneity in size vigorous and were transplanted in November in the first and second seasons in rows 50 cm apart with intra-row spacing of 30 cm. Root soaking treatments: the roots of seedlings were soaked for 30 min in a solution with different concentrations of citric acid before transplanting. Soil application treatments: the roots of plants received various citric acid concentrations after 2 weeks from transplanting. All agricultural practices were applied as commonly recommended for commercial lettuce production in the district.

Recorded Parameters:

Morphological parameters:

At harvest time, all of the vegetative growth and yield parameters of lettuce were measured. Random heads of lettuce were selected from each treatment to check the parameters. The data were recorded for total plant weight (kg), head weight (kg), and root fresh weight (g).

Chemical analysis:

At the end of the season, random leaf tissues were taken from the inner leaves of lettuce heads to conduct chemical analyses. The samples were dried in an oven at 68 °C for 48 hour and crushed to pass through a 1 mm mesh screen, and the (0.1 g) dehydrated sample was digested till a colorless solution was observed, and the volume (50 ml) was maintained with deionized water and filtered and used for the determination of nutrient ions. Mineral ions were determined according to (Parkinson and Allen, 1975).

Determination of nitrate and ammonium content in leaves:

Salicylic acid techniques, as outlined by (Du et al., 2017) were used to measure the NO_3^-. The NH_4^+ levels, were measured by the phenol–hypochlorite technique (Solorzano, 1969).

Reactive oxygen species (ROS) in leaves:

Malondialdehyde (MDA) content: fresh tissues (0.5 g) were homogenized in 5 ml of 0.1% (w/v) TCA solution and centrifuged at 12,000×g for 15 minutes at 4 ˚C. The supernatant and 4 ml (0.5%) of thiobarbituric acid (TBA) (prepared in 20% TCA) were applied. The mixture was heated in a water bath at 95 ˚C for 30 minutes, then cooled and centrifuged at 7500×g for 5 min, and the readings at 532 and 600 nm were obtained using a spectrophotometer; the of MDA was estimated according to (Cakmak and Horst, 1991 and Perveen et al., 2017).

Hydrogen peroxide (H_2O_2) concentration: H_2O_2 content was estimated by homogenizing leaf segments in cold 80% (v/v) acetone in the ratio of 0.1 g tissue to 2 ml of acetone. Homogenates were centrifuged at 12000×g for 10 min at 4 ˚C. The H_2O_2 content was measured spectrophotometrically by monitoring the absorbance at 410 nm following the procedure of (Patterson et al .,1984 and Shah, 2011).

Antioxidant enzymes activity assay in leaves:

Potassium phosphate buffer (100 mM, pH 7) containing 0.1 mM EDTA and 1% polyvinyl pyrrolidone (PVP) (W/V) was used to homogenize 500 mg of lettuce inner leaf tissues at 4 °C. For every gram of plant material, 4 milliliters of buffer were used in the extraction process; the homogenate was centrifuged at 15,000 × g for 15 minutes at 4 °C (Ibrahim and Abdellatif 2016). The activities of superoxide dismutase (SOD), peroxidase (POD), catalase (CAT), polyphenol oxidase (PPO), and phenylalanine ammonia-lyase (PAL) were estimated using the supernatant, which was regarded as an enzyme crude extract.

The SOD was evaluated following (Perveen et al., 2017). When compared to the blank (without enzyme extract), one unit of SOD activity was thought to be equal to the quantity of enzyme that results in 50% inhibition of nitroblue tetrazolium (NBT) photoreduction. The reaction solution contained 50 mM phosphate buffer (pH 7.8), deionized water, NBT (50 lM), methionine (13 mM), enzyme extract (50 µl), and riboflavin (1.3 µl). The reaction mixture was reserved under 15 fluorescent lamps for 15 minutes, and the reading was taken at 560 nm with a spectrophotometer.

Related to the activities of POD and CAT, the enzymes were determined on a protein basis according to (Britton and Mehley 1955) method. In respect of POD, the reaction solution containing distilled water, 100 µl of 40 mM H_2O_2, 250 µl of 50 mM phosphate buffer (pH 7.8), 100 µl of 20 mM guaiacol, and 50 µl enzyme extract was used. The change in POD activity was determined every 20 second at 470 nm in the spectrophotometer.

For the determination of CAT activity, the reaction solution containing 1 ml of H_2O_2 (5.9 mM), 1.9 ml of 50 mM phosphate buffer (pH 7.00), and 100 µM supernatant was used. The difference in absorbance of the reaction mixture was read after 20 second at 240 nm in the spectrophotometer.

PPO was evaluated following (Benjamin and Montgomery, 1973). One unit of enzyme activity was defined as the total amount of PPO enzyme activity that led to a rise in absorbance of 0.001/minute at 420 nm.

The methodology of (Beaudoin-Eagan and Thorpe 1985) was used to estimate PAL enzyme activity. One unit of PAL enzyme activity was defined as the total amount of PAL activity that produced an enhancement in absorbance of 0.01/h at 290 nm.

Statistical analysis:

The effects of the treatments were tested using one-way analysis of variance (ANOVA) with three replicates per treatment. Data were analyzed using CoStat software program (Version 6.400). Means were compared between the treatments using the LSD (least significant difference) test at the 0.05 probability level.

RESULTS

Effect of citric acid on lettuce growth:

All vegetative traits were magnificently affected by the various applications of citric acid in the two seasons. Both soaking and soil application of CA treatments significantly increased lettuce growth characters. The data revealed that the exogenous rates of CA, except T9, significantly enhanced the weights of total plants, heads, and roots when compared with the control in the first and second seasons. When the lettuce growth was measured as the produced biomass, results showed that lettuce produced more plant biomass in season one than in season two. The effects of CA treatments on growth parameters of lettuce plants are presented in **Fig. (1)**.

Effects of citric acid on lettuce head content of nutrients:

Data in **Fig. (2)** showed that the application of CA in two seasons had a highly significant increase of P/K/Ca/Mg concentrations (mg/g dry weight^{-1}) in tissue of lettuce heads, but T9 only appeared to be reduced. Generally, it could be concluded that the highest concentration of P, K^+, Ca^{+2}, and Mg^{+2} was obtained from T7 and recorded (0.58 and 0.75), (5.77 and 5.67), (0.49 and 0.44), and (0.56 and 0.41) during 2022 & 2023, respectively. On the contrary, the T7 recorded the lowest concentration of Na^+ and Cl^-, whereas T9, followed by T10, recorded the highest rates of these two elements in the first and second seasons.

Regarding K^+/Na^+ ratio in lettuce leaves, T7 had the highest level, whereas T9 and control showed the lowest levels **Fig. (3).**

Effects of citric acid on zcontent of NO_3^- and NH_4^+:

The NO_3^- and NH_4^+ levels in leaves exhibited significant differences among the treatments. Under the control and T9 treatments, the accumulation of NO_3^- in leaves greatly increased, although T7 exhibited the lowest value compared to all other treatments in both seasons. Concerning the NH_4^+ content, it was also markedly elevated when plants subjected to T9 and the control compared with other treatments in 2022 and 2023. Due to the lowest NO_3^- which was produced significantly on the leaves by T7, the lettuce leaves exhibited the lowest NH_4^+ **Fig. (4)**.

Effects of citric acid on reactive oxygen species ROS (MDA and H_2O_2), in leaves:

The concentrations of MDA and H_2O_2 **Fig. (5)** showed significant increases in the T9 and the control treatments compared with other treatments. In contrast, the other treatments reduced the accumulation of MDA and H_2O_2 activity in the two seasons compared with the control. The ROS had a highly significant reduction when exposed to T7 compared with all other treatments.

Effects of citric acid on antioxidant enzymes activities in leaves:

The data in **Fig. (6)** showed that the enzyme activities of superoxide dismutase (SOD) and peroxidase (POD) were significantly affected by different treatments. The treatments T7 followed by T8 magnificently increased the SOD and POD activities in 2022-2023 compared with others. Differently, the catalase (CAT) activity had no clear effect from T7compared with T8 and control in the first season, while the CAT had a highly significant increase compared with control in the second season.

Effects of citric acid on polyphenyl oxidase (PPO) and phenylalanine ammonialyase (PAL), in leaves:

The application of T7 showed a highly significant increase in PPO and PAL activities in comparison to untreated plants and all other treatments. The increase in polyphenyl oxidase (PPO) and phenylalanine ammonia-lyase (PAL) activities was higher in the first season than in the second season **Fig. (7)**.

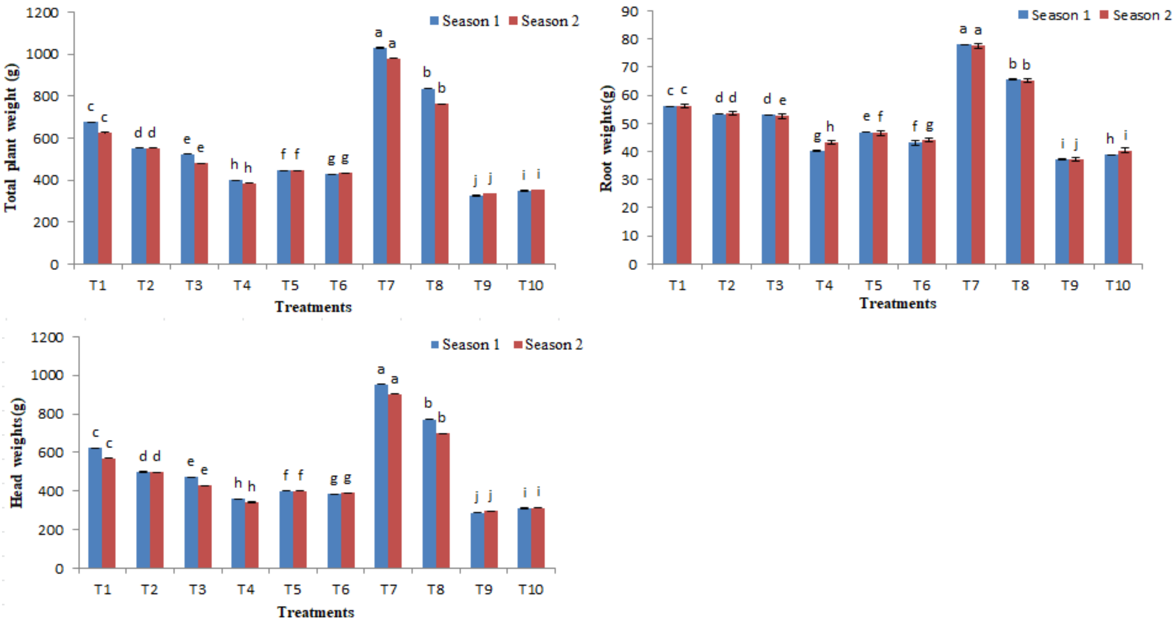
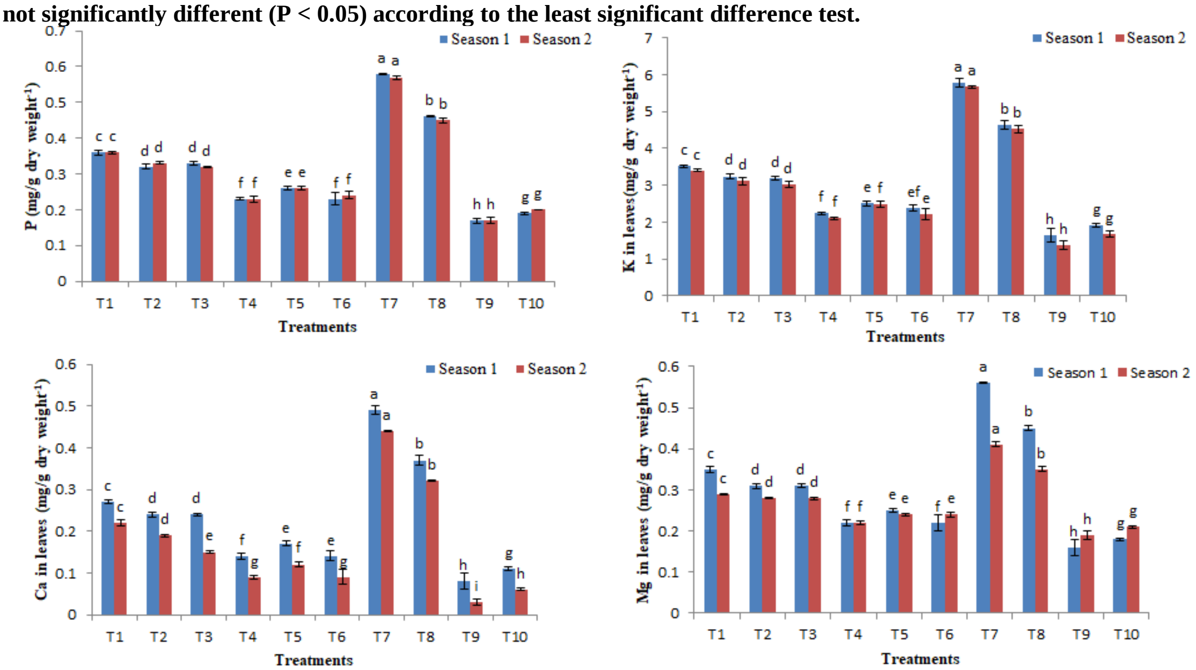


Figure (1): Effect of citric acid application on total yield, head weights and root weights on lettuce plants. T1 = root soaking (RS) 0.5 g/L, T2 = (RS) 0.75 g/L, T3 = (RS) 1 g/L, T4 = soil application (SA) 0.5 g/L, T5 = (SA) 0.75 g/L, T6 = (SA) 1 g/L, T7 = (RS) 0.5 g/L + (SA) 0.5 g/L, T8 = (RS) 0.75 g/L + (SA) 0.75 g/L, T9 = (RS) 1 g/L + (SA) 1 g/L, and T10 = untreated (control). Vertical bars denote the SD. Columns in figure that are headed with the same letter are not significantly different ($P < 0.05$) according to the least significant difference test.



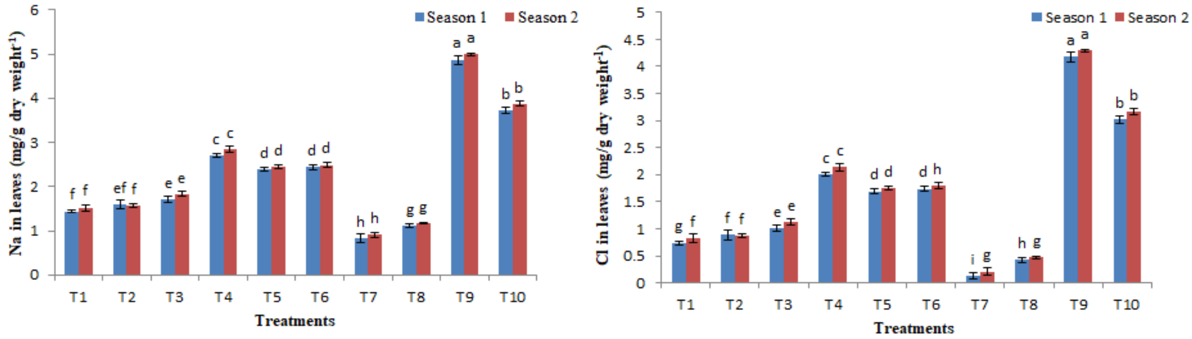


Figure (2): Effect of citric acid application on phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), sodium (Na) and chloride (Cl) in lettuce leaves. T1 = root soaking (RS) 0.5 g/L, T2 = (RS) 0.75 g/L, T3 = (RS) 1 g/L, T4 = soil application (SA) 0.5 g/L, T5 = (SA) 0.75 g/L, T6 = (SA) 1 g/L, T7 = (RS) 0.5 g/L + (SA) 0.5 g/L, T8 = (RS) 0.75 g/L + (SA) 0.75 g/L, T9 = (RS) 1 g/L + (SA) 1 g/L, and T10 = untreated (control).Vertical bars denote the SD. Columns in figure that are headed with the same letter are not significantly different (P < 0.05) according to the least significant difference test.

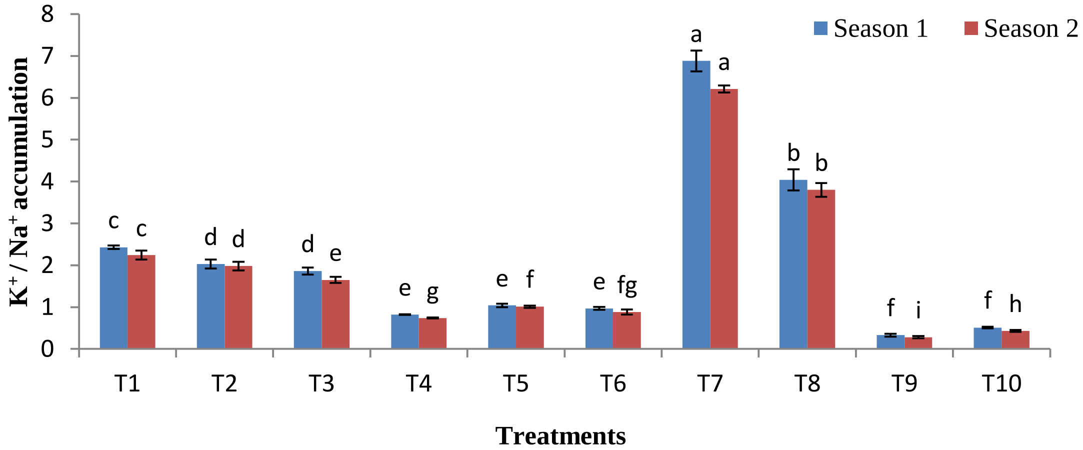


Figure (3): Effect of citric acid application on K+/Na+ in lettuce leaves. T1 = root soaking (RS) 0.5 g/L, T2 = (RS) 0.75 g/L, T3 = (RS) 1 g/L, T4 = soil application (SA) 0.5 g/L, T5 = (SA) 0.75 g/L, T6 = (SA) 1 g/L, T7 = (RS) 0.5 g/L + (SA) 0.5 g/L, T8 = (RS) 0.75 g/L + (SA) 0.75 g/L, T9 = (RS) 1 g/L + (SA) 1 g/L, and T10 = untreated (control). Vertical bars denote the SD. Columns in figure that are headed with the same letter are not significantly different (P < 0.05) according to the least significant difference test.

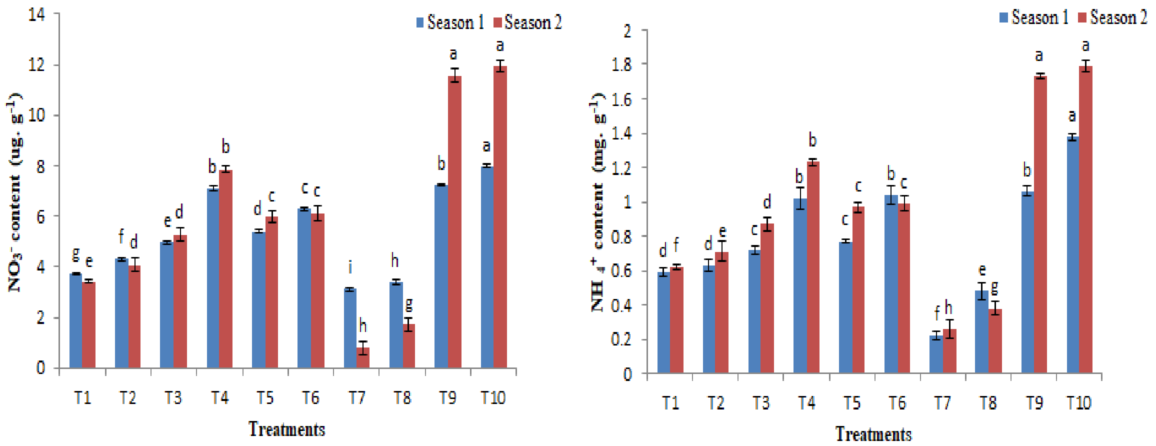


Figure (4): Effect of citric acid application on NO3- and NH4+ in lettuce leaves. T1 = root soaking (RS) 0.5 g/L, T2 = (RS) 0.75 g/L, T3 = (RS) 1 g/L, T4 = soil application (SA) 0.5 g/L, T5 = (SA) 0.75 g/L, T6 = (SA) 1 g/L, T7 = (RS) 0.5 g/L + (SA) 0.5 g/L, T8 = (RS) 0.75 g/L + (SA) 0.75 g/L, T9 = (RS) 1 g/L + (SA) 1 g/L, and T10 = untreated (control). Vertical bars denote the SD. Columns in figure that are headed with the same letter are not significantly different (P < 0.05) according to the least significant difference test.

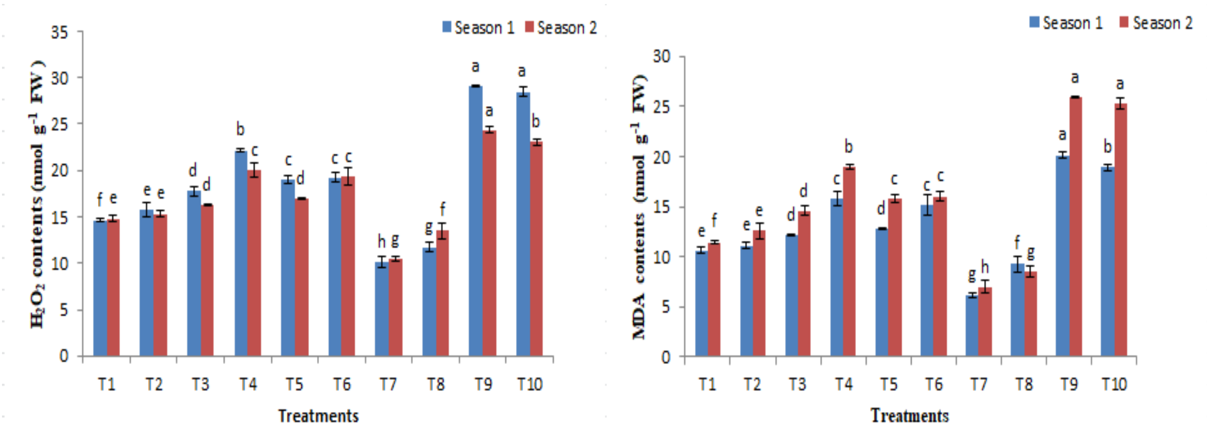


Figure (5): Effect of citric acid application on H2O2 and MDA content in lettuce leaves. T1 = root soaking (RS) 0.5 g/L, T2 = (RS) 0.75 g/L, T3 = (RS) 1 g/L, T4 = soil application (SA) 0.5 g/L, T5 = (SA) 0.75 g/L, T6 = (SA) 1 g/L, T7 = (RS) 0.5 g/L + (SA) 0.5 g/L, T8 = (RS) 0.75 g/L + (SA) 0.75 g/L, T9 = (RS) 1 g/L + (SA) 1 g/L, and T10 = untreated (control). Vertical bars denote the SD. Columns in figure that are headed with the same letter are not significantly different (P < 0.05) according to the least significant difference test.

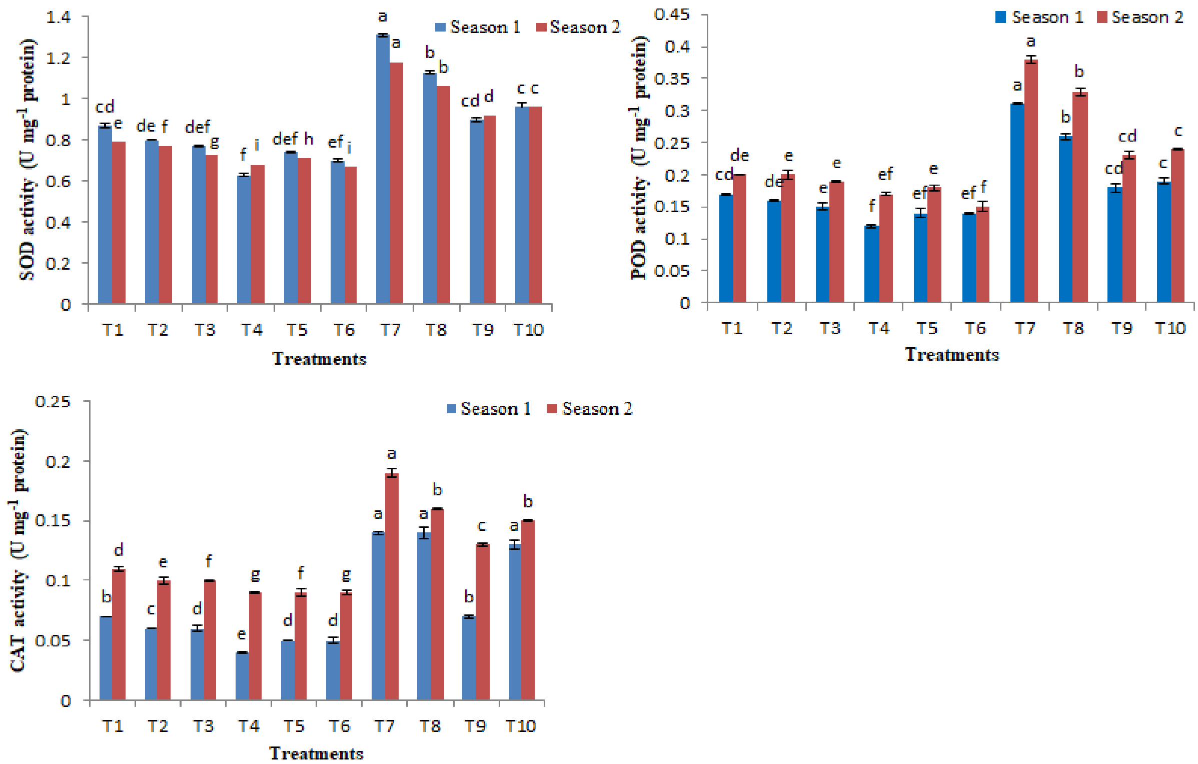


Figure (6): Effect of citric acid application on antioxidant enzyme activities of superoxide dismutase (SOD), peroxidase (POD), and catalase (CAT) in lettuce leaves. T1 = root soaking (RS) 0.5 g/L, T2 = (RS) 0.75 g/L, T3 = (RS) 1 g/L, T4 = soil application (SA) 0.5 g/L, T5 = (SA) 0.75 g/L, T6 = (SA) 1 g/L, T7 = (RS) 0.5 g/L + (SA) 0.5 g/L, T8 = (RS) 0.75 g/L + (SA) 0.75 g/L, T9 = (RS) 1 g/L + (SA) 1 g/L, and T10 = untreated (control). Vertical bars denote the SD. Columns in figure that are headed with the same letter are not significantly different (P < 0.05) according to the least significant difference test.

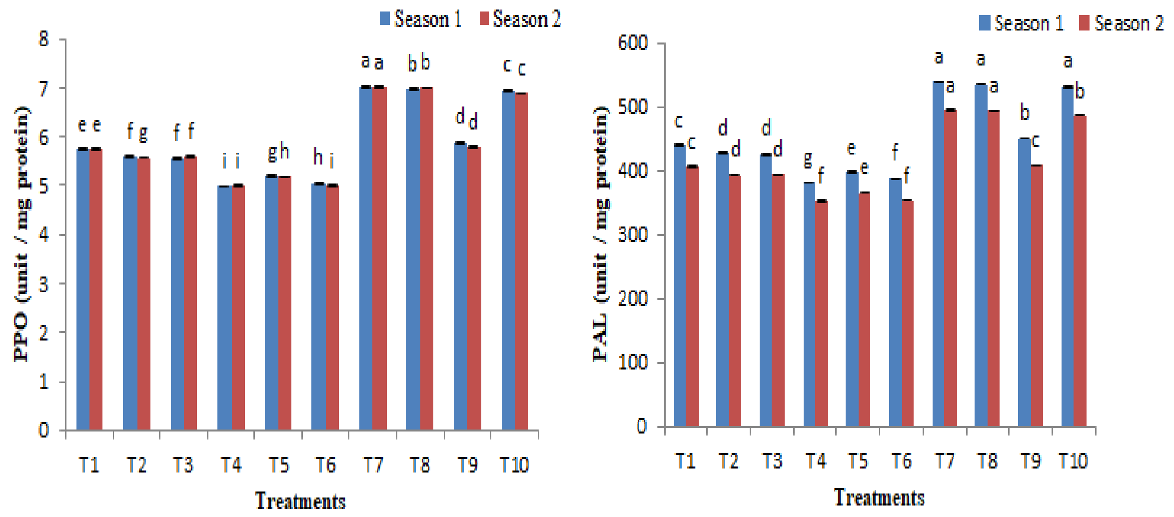


Figure (7): Effect of citric acid application on polyphenyl oxidase (PPO) and Phenylalanine ammonialyase (PAL) activities in lettuce leaves. T1 = root soaking (RS) 0.5 g/L, T2 = (RS) 0.75 g/L, T3 = (RS) 1 g/L, T4 = soil application (SA) 0.5 g/L, T5 = (SA) 0.75 g/L, T6 = (SA) 1 g/L, T7 = (RS) 0.5 g/L + (SA) 0.5 g/L, T8 = (RS) 0.75 g/L + (SA) 0.75 g/L, T9 = (RS) 1 g/L + (SA) 1 g/L, and T10 = untreated (control). Vertical bars denote the SD. Columns in figure that are headed with the same letter are not significantly different (P < 0.05) according to the least significant difference test.

DISCUSSION

Our results showed a significant growth increase of lettuce morphological parameters when implicated with citric acid via root soaking plus soil application (T7). Du et al. (2017) reported that significant alterations to a plant's ecology and physiology are intimately linked to its roots. The root is the first organ affected and receives nutrients and water from the soil by root hairs. Therefore, our study was aimed at dealing with the lettuce roots by CA soaking treatments before transplanting to replace root hairs lost during separation from the trays. This process may rapidly generate an extensive root system in the soil, hence decreasing the duration required to restore the root-to-shoot ratio post-transplantation and enabling the lettuce to obtain nutrients and water from the soil. Accordingly, the treated roots with citric acid (CA) increased the growth of lettuce; this increase may be by modifying major metabolism, such as photosynthesis process, which produces the chemical energy needed for lettuce development, growth, and reproduction (Chen et al., 2013).

Additionally, the aim of using organic materials is to promote root and overall plant growth, which will enhance the ability to absorb available nutrients (Papenfus et al., 2013). In contrast to the control, our study found that applying T7 greatly increased the P, K^{+}, Ca^{2+}, and Mg^{2+} of lettuce leaves and significantly decreased the Na^{+} element. The greatest growth-restrictive factors on plant growth are nutrients, which also alleviate the hurtful effects of abiotic stress by reducing the uptake of Na^{+} and Cl^{-} by plants (Grattan and Grieve 1999., Umar et al., 2011). Na^{+}, Ca^{2+}, and Mg^{2+} are common cations linked to salinity, whereas Na^{+} and Cl^{-} ions are thought to be the most significant because they are harmful to plants and, in particular, reducing cellular metabolism and deteriorate the physical structure of the soil (Umar et al., 2011). In this study, the growth reduction of lettuce plants may be related to the soil secondary salinization. He et al. (2007) reported that excessive chemical fertilizer insufficient irrigation, a long period of intensive farming, and many other factors have triggered a severe problem of secondary salinization $Ca(NO_3)_2$ (Yu et al., 2005). It is well known that the $Ca(NO_3)_2$ resulted from the reaction between $Ca^{2+,}$ that presented about 60% of the total cations, and

NO_3^-, which counts for nearly 67–76% of the total anions (Du et al., 2017). $Ca(NO_3)_2$ destroys the osmotic homeostasis potential of plant cells, leads to nutrient stress, and causes the inhibition of lettuce development. Our suggestion that the high significant increase of nutrient uptake, which is considered the most important primary metabolism, could alleviate secondary salinization in lettuce leaves (Ahmad Parvaiz et al., 2014).

Regarding to K^+/Na^+ ratio, our results illustrated that the superiority of T7 compared with the control and other treatments. The plant mechanism to sustain K^+/Na^+ homoeostasis is crucial and a very important approach for tolerance under stressful conditions (Hasegawa et al., 2000 and Siddiqui et al., 2012). A similar trend has been observed in this study with respect to K^+ and Na^+ accumulation in lettuce leaves. Application of T7 stimulated the cumulating of essential nutrients, which is an important indicator of dry matter productivity because they are components of numerous metabolically important compounds and assistance in biological and physiological functions (Marschner 2011).

During our study, we observed that a large amount of NO_3^- accumulated in lettuce leaves by T10, followed by T9, and severely inhibited lettuce growth. Our investigation could suggest that the citric acid-induced nitrogen enzymes metabolism in lettuce leaves. NO_3^- is a principal nitrogen source for plants (Delgado et al., 1994). The dramatic accumulation of NO_3^- under salt stress in lettuce leaves might be that the preliminary stages of N metabolism in plants are highly sensitive to abiotic stress (Debouba et al., 2007). This disturbance under abiotic stress of NO_3^- that could arise in the plants because of antagonistic homeostasis NO_3^-/Cl^- (Khan and Srivastava 1998). The current results were in agreement with those that were found in tomato (Debouba et al., 2007) and pigeon pea (Dalio et al., 2013). Ammonium represents the ultimate state of mineral nitrogen before the formation of organic nitrogen molecules (Shao et al., 2015). The increase of NH_4^+ by T10 and T9 may result from the rate-limiting phase in assimilation, which involves the conversion of NO_3^-, after cellular uptake, to NH_4^+ by nitrogen enzymes (Du et al., 2017 and Yuan et al., 2012). The current investigation demonstrated a significant accumulation of NH_4^+ in leaves under the control treatment. Yang et al. (2013) noticed accumulation of NH_4^+ under abiotic stress in watermelon, which was strictly associated with ammonia absorption; the disturbance can inhibit crops growth due to the toxic effect of NH_4^+. Our suggestion is that the accumulation of excessive amounts of endogenous NH_4^+ in plant organs is detrimental and can negatively affect protein creation, cytosolic pH, and coupling of photophosphorylation in higher plants (Kronzucker et al., 2001). Debouba et al. (2007) and Shao et al. (2015) reported that a higher rise in ammonium concentration is accompanied by a higher fall in soluble protein content, which has been linked to slower growth.

Reactive oxygen species (ROS) can diminish the growth and productivity of most crops. Our result suggested that the application of T7 to lettuce root reduced ROS (MDA and H_2O_2) compared with all other treatments. Our suggestion is the increase of ROS activity by other treatments that unfavorable and toxic nutrients may be increased in the soil and water. That increase could cause oxidative stress and inhibit plant growth by accelerating the production of reactive oxygen species and lipid peroxidation (Najeeb et al., 2009). Moreover, stress induces injuries to compatible solutes in leaves and eventually to cellular structure via cytotoxic ROS productivity. In addition, the increase of H_2O_2 accumulation causes membrane damage, which accelerates MDA production. MDA is used as an ideal indicator of lipid peroxidation, which causes increased membrane permeability coefficient, fluidity, and cause a reduction in growth (Ibrahim and Abdellatif, 2016).

Accordingly, the plant activates a defensive mechanism by motivating a number of antioxidant enzymes that show a vital action in scavenging (ROS) (Simova-Stoilova et al., 2008).

Our research focuses on the antioxidant enzymes defense system of plants, which includes (SOD), (POD), and (CAT). The present study shows that the activities of these enzymes obviously augmented when lettuce plants were exposed to T7 rather than control plants among the various CA concentrations applied. SOD is the first line that protects plants from oxidative destruction by transforming O_2^- (superoxide anion) into H_2O_2. Similarly, POD is a key enzyme that causes the delay, breakdown and removal of H_2O_2 also, it is known to protect cells from ROS by stimulating redox processes. While, CAT is localized in the different cell parts, which reclaims ROS directly by converting H_2O_2 to water and oxygen (Ahmad Hussein et al., 2018 and Mittler , 2002). In the first season, the CAT had no significant effect and had a highly magnificent increase in the second season compared with the control. It may be related to the factors that supplemented abiotic stress accumulation increased in season two more than in season one. Hence, we suggest that the role of SOD and POD is more effective than CAT to complete the scavenged ROS, or the lettuce didn't need to activate the CAT under the low-stress season. Citric acid treatment boosted the activity of certain anti-oxidative enzymes, indicating that lettuce plants were more resistant to abiotic stress-induced oxidative bursts by using 0.5 g root soaking + 0.5 g soil application of CA.

Our results reported that the application of CA to lettuce root had a highly magnificent induction of PAL and PPO. Regarding PAL, T7 had no difference between T8 and showed difference among control and other treatments. Our study discussed that, PAL is a key enzyme for producing phenylpropanoids by deamination of the amino acid l-phenylalanine. Phenylpropanoids influence many sides of plants reactions to environmental stressful conditions, like mineral stress (Chen et al., 2013 and Lee et al., 2004). This research indicates that phenylalanine biosynthetic pathways are critical for the biosynthesis of organic acids such as citric acid and/or salicylic acid under stressful conditions. PAL activities reflected the rate-limiting enzyme under secondary metabolism (Dixon and Paiva 1995). Also, PAL enzyme is important in the phenylpropanoids pathway (Ibrahim and Abdellatif, 2016).

On the other side, PPO enzyme had a high increase by T7 compared with other treatments. PPO, which is found in plants, is believed to be essential for protecting them from environmental threats (Chen et al., 2013 and Choudhary et al., 2009). In this regard, our results are consistent with previous research suggesting that PPO catalyzes the oxidation of phenols. Mayer (2006) demonstrated that PPO activity may regulate the levels of phenolic compounds and perform a role in the phenylpropanoid pathway.

The study of the antioxidant enzymes SOD, POD, CAT, PAL, and PPO revealed that activities of these enzymes were affected by soaking + soil application of citric acid, and this was especially dependent on the citric acid concentrations submission. These obtained results might be attributed to the potential effects of citric acids which act as free radical scavenger.

CONCLUSION

Our study could conclude that the untreated plants with citric acid decreased the growth of lettuce by generating reactive oxygen species (ROS), decreasing the K^+/Na^+ ratio, and accumulating NO_3^- and NH_4^+ as well as antioxidant enzymes in leaves. On the other hand, additions of citric acid magnificently alleviate the harmful effect of salinity by modifying the K^+/Na^+ ratio, reducing ROS, and promoting antioxidant activities in lettuce. Citric acid application (root soaking 0.5 g/L + soil application 0.5 g/L) was found more effective in alleviating the perilous effect of aged soil secondary salinization. In relation to these criteria, this dose had a highly significant effect when compared to other treatments. Our investigation shows that applying citric acid, an organic input, can decrease the buildup of NO_3^- and NH_4^+ in lettuce leaves, making the crop healthier and high quality.

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الملخص العربي

تحسين النظام الدفاعي لنباتات الخس عن طريق المعاملة الجذرية بحامض الستريك

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تمثل الممارسات الزراعية الخاطئة عبر العقود خطورة كبيرة علي إنتاجية المحاصيل المختلفة. وبناءً عليه فإن هناك حاجة ماسة لإجراء بعض المعاملات التي تساعد النبات في مجابهة هذه المشكلة. أثبتت الأبحاث السابقة أن حامض السيتريك أحد أهم الأحماض العضوية التي تساعد علي النمو السريع للجذور حيث أنه ينتج عن طريق الجذور بصورة طبيعية. صممت هذه الدراسة لإختبار كفاءة الإضافات الجذرية لحامض الستريك علي محصول الخس لموسمي (٢٠٢٢– ٢٠٢٣) لتدعيم الجذور عن طريق معاملات النقع (نصف ساعة) قبل إجراء عملية الشتل بالأرض المستديمة, ولتقليل الآثار السلبية عن طريق معاملات الإضافة للتربة بعد أسبوعين من الزراعة. أجريت هذه الدراسة علي نباتات خس الكابوتشا صنف (بيج بيل), وكانت المعاملة بحامض الستريك كالتالى: معاملات نقع الجذور قبل الشتل T1= 0.5 جم/ لتر, T2= 0.75 جم/ لتر , T3= 1جم/ لتر, معاملات الإضافة للتربة T4= 0.5 جم/ لتر, T5= 0.75 جم/ لتر, T6= 1 جم / لتر, معاملات نقع للجذور + معاملات الإضافة للتربة T7= 0.5 جم/ لتر+ 0.5 جم/ لتر, T8= 0.75 جم/ لتر+ 0.75 جم/ لتر, T9= 1 جم/ لتر+1 جم/ لتر, T10= نباتات غير معاملة (كنترول). في كلا الموسمين أظهرت النتائج إستجابة ملحوظة في صفات النمو الخضرية عند المعاملة بحامض الستريك. حيث أظهرت جميع المعاملات إيجابية عالية مقارنة بالكنترول ما عدا المعامله T9 التي أظهرت إنخفاض مقارنة بالكنترول. كما أظهرت المعامله T7 إيجابية عالية مقارنة بجميع المعاملات الأخرى. مما سبق نستنتج أن لحامض الستريك دور فعال في تقليل الآثار الضارة للإجهاد اللاحيائى وذلك عن طريق تقليل تراكم المواد الضارة بالأوراق وأيضا عن طريق تحفيز نشاط الإنزيمات المضادة للأكسدة. من الممكن أن توصي هذه الدراسة بإستخدام حامض الستريك بمعدل 0.5 جم/ لترنقعا للجذورلمدة نصف ساعة قبل الشتل + 0.5 جم/ لتراإضافة للتربة بعد الزراعة بأسبوعين علي محصول خس الكابوتشا صنف بيج بيل للحصول علي أعلي إنتاجية .