## THE ROLE OF SELENIUM IN MITIGATING THE HARMFUL EFFECTS OF ALKALINITY STRESS IN TOMATO SEEDLINGS

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Abstract. Tomato is considered one of the most popular vegetables worldwide playing a vital role in human nutrition. Alkalinity is common environmental stresses which limits agricultural crop production. In order to investigate the impact of trace elements of selenium on agro-physiological features of tomato under alkaline stress conditions, two experiments were organized as a randomized complete block design and each treatment four replications were conducted in the greenhouse of Horticultural Department, Faculty of Agriculture, Ain-shams University, Egypt. The experimental groups were the followings: (1) untreated plants (control), (2) plant treated with of 75 mM sodium carbonate (Na<sub>2</sub>CO<sub>3</sub>), (3) plants treated with selenium (Se, at rate of 5  $\mu$ M and (4) plants treated with selenium and sodium carbonate. the obtained results showed that the exogenous application of Se caused a significant improvement in shoot biomasses, leaf photosynthetic pigments (Chlorophyll a, b, total and carotenoids), and leaf relative water and leaf proline content compared to untreated plants, under normal and alkaline stress conditions. Under alkaline stress conditions, application of Se significantly increased the activity levels of leaf antioxidant enzymes (SOD, CAT, POX and APX) when compared with control treatment. On the contrary, higher reduction in the sodium content (Na), Na/K ratio, hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>), malondialdehyde (MDA) and membrane stability index (MSI) was observed in the leaves of tomato plants sprayed with Se more than untreated ones. In both conditions, application of selenium increased the potassium content (K) in the plant leaves more than that in untreated plants. Based on the results, selenium applications could be suggested as promising treatment to alleviate adverse effects of alkalinity stress on agro-physiological and biochemical characteristics.

Keywords: Solanum lycopersicum, trace elements, enzyme activity, growth performance, plant tolerance

#### Introduction

Soil alkalinity is a global problem that predominates in arid and semi-arid areas and harms human health and food security (Moradbeygi et al., 2020; Javed et al., 2022). Soil sodicity is mostly caused by low rainfall, over-application of chemical fertilizers, irrigation of underground brackish water, industrial wastes, and deforestation (Mahmoud et al., 2022; Hashimi and Habibi, 2021). All the previous factors upsurge of exchangeable sodium content around the root zone of a soil profile. Higher concentrations of exchangeable sodium worsen the physicochemical, and biological characteristics of soil, eventually causing a reduction in the quantity and quality of economic crops (Mahmoud et al., 2019; Mushtaq et al., 2021). Meanwhile, higher accumulation of sodium ions in plants stimulates oxidative injury through the generation of reactive oxygen species (ROS) (Abdeldaym et al., 2020; Hasanuzzaman et al., 2020) that can cause damage to cell membranes, protein biosynthesis, and carbohydrate metabolism (Vwioko, et al., 2019; Rajabi Dehnavi et al., 2020). Generally, agriculture on Sodic-degraded soils has always remained a challenge due to the multidimensional impacts of alkalinity on soils and plants (Kumar et al., 2009; Rizwan et al., 2023).

In order to decrease the damaging impacts and scavenge the ROS, plants have enzymatic and non-enzymatic antioxidant defense systems in their cells (Abdelaziz et al., 2021; Aung et al., 2022; Shehataet al., 2022; Mahmoud et al., 2023). Modifications in the activity level of antioxidant enzymes have been observed in different abiotic stresses. Furthermore, the obliteration of proteins and the accumulation of some free amino acids for the conservation and modulation of osmotic stress of the cells were mentioned under the same conditions (Hissao, 1973; EL-Beltagi et al., 2023). Lately, several physicochemical and biological approaches have been suggested for the sustainable production of products in sodic soils (Mousavi et al., 2022). Among all these strategies, foliar nutrient application is an effective method to alleviate the adverse effects of salinity or alkalinity stress as well as improve plant growth and production (Mahmoud et al., 2019; Mousavi et al., 2022; Hassan et al., 2023; El-Beltagi et al., 2023; Rizwan et al., 2023).

Among the mineral nutrient, selenium is known as a trace element for plants but vital for human beings (Bybordi, 2016). Selenium upsurges the tolerance of plants to environmental stresses via increasing the activity level of antioxidant content in stressed plants, and thus proven to be effective in the growth and development of different plants (Sousa et al., 2023). Growth performance, photosynthetic activity, photosynthetic pigments and antioxidant content have improved in stressed plants exposed to the exogenous application of selenium (Tang et al., 2023). This application has protected the cell membranes and enhance the tolerance of stressed plants (Hawrylak-Nowak, 2009). Other study on tomato seedlings showed that vegetative growth parameters and photosynthetic activity of salt stressed plants increased by application of selenium (Mousavi et al., 2022). KeLing et al. (2013), reported that application of selenium improves growth performance, increase in the activity of antioxidant enzymes and reduced malondialdehyde content of stressed melon plants. Therefore, this study aimed to investigate the impact of exogenous selenium application on agro-physiological and chemical properties of tomato plants.

## Material and methods

#### Plant materials and treatments

Tomato (*Solanum lycopersicum* L. 023 F1) produced by Sakata Vegetables, Europe, France, seeds were sterilized with 0.5% NaOCl (w/v) for 4 min and washed with distilled water for 5 times, and germinated at 25°C in the dark on filter paper in petri dishes. Germinated seeds were then transplanted individually in 13 cm in diameter plastic pots filled with peat and vermiculite (3/1 v/v). Seedlings were grown in growth chamber under the following conditions: 400 µmol m<sup>-2</sup> s<sup>-1</sup> light density with photoperiod 14 h, 25/20°C day/night temperature and 60% of relative humidity. At the four-leaf stage, tomato seedlings were irrigated for a week one time daily with 150 ml of half-strength Hoagland nutrient solution with or without 75 mM Na<sub>2</sub>CO<sub>3</sub> to apply the alkalinity stress. At the same time, selenium (Se) was added as sodium selenate (Na<sub>2</sub>SeO<sub>4</sub>) at 5 µM. The total treatments were four as follow: (1) control without Se or alkaline stress, (2) Alkaline stress without Se, (3) Se without alkaline stress, and (4) Alkaline stress with Se. The experiment was arranged in a complete randomized design with three replicates.

#### Determination of plant growth and photosynthetic pigments

Three days after the last application with alkalinity and Se, tomato seedlings were collected and washed with distilled water. Shoots and roots were separated and

weighed, respectively. Chlorophyll a, b and total chlorophyll was determined in the acetone extract using two specific wavelengths at 645 and 662 nm as described by Costache et al. (2012) Carotenoids were quantified using the acetone and petroleum ether method as described by de Carvalho et al. (2012) using the following formula:

Carotenoids (mg/g FW) = 
$$\frac{A 450 \text{ x V (mL) x 10}}{A1\%1 \text{ cm x W (g)}}$$
 (Eq.1)

where A450 = absorbance at 450 nm; V = total extract volume; W = sample weight; A1%1 cm = 2592 ( $\beta$ -carotene coefficient in petroleum ether).

#### Determination of proline and relative water content

Proline was measured using the ninhydrin reagent as described by Bates et al. (1973). Leaf relative water content (RWC) was determined according to Sattar et al. (2023).

#### Determination of antioxidant enzyme activities

Leaves of tomato were weighed accurately (0.3 g fresh weight) and ground with 3 mL chilled 50 mM potassium phosphate buffer (pH 7.8) containing 0.2 mM ethylene diaminetetraacetic acid (EDTA), 2 mM ascorbate and 2% polyvinylpyrrolidone (PVP). Eachhomogenate was centrifuged at 4°C for 20 min at 12,000 g and thesupernatant was used for determination of antioxidant enzymeactivities.

The method described by Beyer Jr and Fridovich (1987) was used to assess the activity of superoxide dismutase (SOD, EC 1.15.1.1). In summary, a portion of the enzyme was exposed to both light and darkness to observe the reduction of nitroblue tetrazolium at a wavelength of 560 nm. The activity of superoxide dismutase (SOD) was measured in enzyme units (EU) per milligram of protein. The activity of catalase (CAT, EC 1.11.1.6) was determined using the assay method described by Weydert and Cullen (2010). The change in optical density at 240 nm was measured. A extinction coefficient of  $36 \times 103$  mM-l cm-l was utilized for the calculation and expressed as EU mg-1 protein. To determine the activity of ascorbic peroxidase (APX), 0.1 mL of the enzyme was combined with 1 mL of potassium phosphate buffer (100 mM, pH 7.0), 0.1 mM EDTA, 0.5 mM ascorbate, and 0.1 mM H<sub>2</sub>O<sub>2</sub>. The vanishing of H<sub>2</sub>O<sub>2</sub> was detected through a modification in absorbance at 290 nm (Nakano and Asada, 1981).

#### Determination of membrane stability and oxidative stress markers

0.1 g of fresh leaf tissue was coarsely chopped and placed in test tubes with 10 mL of distilled water to determine the membrane stability index (MSI). Tubes were boiled at 40°C to determine electric conductivity (EC1). The electric conductivity (EC2) was again measured after boiling the tubes at 100°C (Abd Elbar et al., 2021). Formula for MSI percentage: 1 - (EC1/EC2) \* 100. Fresh leaf samples were pestled and mortared in 0.1% TCA to assess H<sub>2</sub>O<sub>2</sub> content. The liquid above the sediment was mixed with 0.5 mL of a solution containing 10 millimoles of potassium phosphate buffer at pH 7.0 and 1 millimole of potassium iodide in a 1 mL volume after 15 min of centrifugation at 12000 rpm. After measuring the mixture's optical density at 390 nm (El-Mogy et al., 2022), an H<sub>2</sub>O<sub>2</sub> standard curve was used to calculate it. Malondialdehyde (MDA) content was assessed as an index of lipid peroxidation using thiobarbituric acid (TBA) by recording absorbance at 535 and 600 nm (Heath and Packer, 1968).

### Quantification of Na and K

The concentration of Na and K were determined using the flame photometric method (Jenway, UK) as described by Havre (1961).

#### Statistical analysis

The statistical analysis was conducted utilizing Tukey's Multiple Comparison test (One-way ANOVA) using SAS software 9.1 for windows;  $p \le 0.05$ . All measurements were presented as means  $\pm$  standard error (SE). All figures were prepared using Microsoft Excel 2010.

#### Results

#### Effect of applied Si application on plant growth

Data in *Figure 1* showed that the applied selenium application significantly improved the shoot and root fresh weight of tomato plants, under normal and alkalinity stress. The maximum values of shoot and root fresh weight were recorded in tomato plants sprayed with Se under normal conditions while the minimum values were observed in tomato plants grown under alkalinity conditions without spraying with Se. The application of Se increased shoot and root fresh weight by 16% and 15.55% under normal conditions and 43.75% and 39.6% under alkaline stress conditions, respectively.



*Figure 1.* Effect of selenium application and alkalinity stress on shoot fresh weight (A) and root fresh weight (B). Means followed by a different letter are significantly different according to Tukey test (p = 0.05). Vertical bar indicates average standard error (n = 3)

#### Effect of Se application on photosynthetic pigments

Exogenous application of Se and alkalinity stress significantly influenced chlorophyll a (Chl. a), chlorophyll b (Chl. b), and total chlorophyll (total Chl.) in the leaves of tomato plants (*Fig. 2*). The highest Chl a, Chl b, and total Chl were recorded in the leaves of tomato plants sprayed with Se than in untreated plants, under normal conditions. Under alkalinity conditions, the maximum values of Chl a, Chl b, and total Chl were observed in leaves of tomato plants treated with Se than untreated ones. On the contrary, the leaf carotenoid content did not show any significant changes among treatments, under both conditions.



**Figure 2.** Effect of selenium application and alkalinity stress on chlorophyll a (A) chlorophyll b (B), total chlorophyll (C) and carotenoids (D). Means followed by a different letter are significantly different according to Tukey test (p = 0.05). Vertical bar indicates average standard error (n = 3)

## Effect of Se application on relative water content and proline

The results shown in *Figure 3* that under the non-stress conditions, no significant changes were found among all treatments and control in leaf relative water content (RWC) and leaf proline content. Under alkaline stress conditions, selenium application significantly increased the values of RWC and proline content compared to soil treated with sodium carbonate (Na<sub>2</sub>CO<sub>3</sub>). The application of Se improved the RWC and proline content of alkalinity-stressed plants by 14.86% and 27.27%, respectively, compared to the control treatment.

## Effect of Se application on antioxidant enzymes

Data in *Figure 4* illustrate that under normal conditions, there were not significant differences among treatments and control in the activity levels of antioxidant enzymes. Under alkaline stress conditions, the highest activity levels of superoxide dismutase (SOD) catalase (CAT), Peroxidase (POX), and ascorbate peroxidase (APX) were recorded in leaves of plants sprayed with Se treatment compared to untreated plants. furthermore, the Se application raised the activity levels of SOD, CAT, POX, and APX in the leaves of alkalinity-stressed plants by 45%, 9.3%, 12.5%, and 49.67%, respectively compared with untreated plants.

# Effect of Se application on membrane stability index, malondialdehyde, and hydrogen peroxidase

Under non-stress conditions, the membrane stability index (MSI) malondialdehyde (MDA), and hydrogen peroxidase (H<sub>2</sub>O<sub>2</sub>) in leaves of tomato plants was not affected by Se application, as presented in *Figure 5A*, *B*, *C*. Under alkalinity stress, the Se application significantly increased value of MSI (5.33%) and reduced the concentrations of MDA and H<sub>2</sub>O<sub>2</sub> compared untreated ones. On the other hand, the highest

concentrations of MDA (29.2%) and  $H_2O_2$  (39.79%) compared were recorded in control treatment (*Fig. 5B, A*).



**Figure 3.** Effect of selenium application and alkalinity stress on chlorophyll a (A) chlorophyll b (B), total chlorophyll (C) and Carotenoids (D). Means followed by a different letter are significantly different according to Tukey test (p = 0.05). Vertical bar indicates average standard error (n = 3)



**Figure 4.** Effect of selenium application and alkalinity stress on superoxide dismutase (SOD, A) catalase (CAT, B), Peroxidase (APX, C) and ascorbate peroxidase (APX, D). Means followed by a different letter are significantly different according to Tukey test (p = 0.05). Vertical bar indicates average standard error (n = 3)

#### Effect of Se application on leaf nutrient content

The absorption of nutrients, especially sodium (Na) and potassium (K) were significantly affected by selenium application, under normal and alkaline stress conditions. The application of Se significantly reduced leaf Na content and increased leaf K content of tomato plants grown in an alkalinity-stressed environment

(*Fig. 6A, B*). A similar trend was observed in the ratio Na/K (*Fig. 6C*). The ratio of Na/K in the leaves of alkalinity-stressed plants was also reduced by Se application compared to untreated plants. Under normal conditions, the application of Se significantly improved leaf K concentration by 22.22% compared to untreated tomato plants. Whereas, there were not significant changes in leaf Na concentration and Na/K ratio in treated and untreated plants (*Fig. 6A, C*).



Figure 5. Effect of selenium application and alkalinity stress on membrane stability index (MSI, A) malondialdehyde (MDA, B), and hydrogen peroxidase ( $H_2O_2$ , C). Means followed by a different letter are significantly different according to Tukey test (p = 0.05). Vertical bar indicates average standard error (n = 3)

## Discussion

Alkalinity stress is considered one of the most important environmental factors that limit sustainable agriculture production worldwide (Aung et al., 2022). Whereas, alkaline stress can reduce growth performance and development of cash crops due to disturbing several physiological processes, including nutrient uptake, photosynthetic activity, ROS balance, and the antioxidant system (Geng et al., 2021). The current study confirmed that soil affected with alkalinity showed a significant reduction in plant biomasses (*Fig. 1*), and chlorophyll content (*Fig. 2*). Similar results were observed by Wei et al. (2015), who reported that alkaline stress significantly reduced root growth, and decreased chlorophyll content, leaf relative water content, and total biomass of rice seedlings. This reduction in chlorophyll content and plant biomass could be related to an increase in the endogenous Na in plant tissues (*Fig. 6*) which induces ROS production such as  $H_2O_2$  (*Fig. 5C*) which finally leads to a severe decrease in the chlorophyll formation, photosynthetic rate and carbohydrate accumulation in different parts of plants. In addition, Aung et al. (2022) confirmed that under alkaline conditions, the expression of a series of genes related to chlorophyll biosynthesis, chloroplast development, carbon fixation, and photosynthesis rate was significantly down-regulated.



Figure 6. Effect of selenium application and alkalinity stress on leaf sodium content (Na, A) leaf potassium content (K, B), and Na/K ratio (C). Means followed by a different letter are significantly different according to Tukey test (p = 0.05). Vertical bar indicates average standard error (n = 3)

On the other hand, the tomato plants sprayed with selenium showed higher root and shoot biomasses than untreated plants, under stressful and non-stressful conditions. Tufail et al. (2023) stated that the application of Se improved the cell division, relative water contents, photosynthetic activity, chlorophyll formation and antioxidant enzyme activities that may improve plant biomasses under environmental stresses conditions.

Furthermore, an increase in the pH of the soil can also cause a reduction in leaf water content (Malekzadeh et al., 2021), as confirmed in this study. Mousav et al. (2022) reported that the leaf-relative water content of cucumber plants grown in alkaline soil was significantly reduced. Leaf relative water content can be considered as an indicator of water status in the different plants, and show the balance between leaf relative water content and transpiration amount (Mahmoud et al., 2022; El-Bauome et al., 2022; Helmy et al., 2024). If the RWC is high, the plant preserves its cellular turgor and continues its growth and development (Rao and Mendham, 1991). In this study showed that the application with selenium caused an improvement in the leaf-relative water content of alkalinity-stressed tomato plants. These findings could be related to the positive effect of

selenium treatments on the improvement of leaf stomatal conductance and transpiration rate under alkalinity conditions. Mousav et al. (2022) mentioned that the exogenous application of Se improves RWC in plants due to enhancing water absorption by the developed root system or decreasing of water loss by transpiration rate.

Likewise, the changes in soil pH conditions induce the production of ROS, such as  $H_2O_2$  (Fig. 5C), in plant cells (Aung et al., 2022). This can damage the cell membranes of plant cell and organelle membranes, due to their high polyunsaturated fatty acids, by inducing the chains of oxidation processes of unsaturated fatty acids and increase the accumulation of malondialdehyde (MDA) in plant cells (Mahmoud et al., 2022). These findings were consistent with the results of the current study, where the plants grown in soil affected by alkalinity showed higher levels of  $H_2O_2$  and MDA (Fig. 5B, C) and lower membrane stability index of plant cells (Fig. 5A). In response to increase ROS production under stressful conditions, plants activate some ROS-scavenging enzymes (SOD, POD, CAT, and APX) and non-enzymatic antioxidants (ascorbate, glutathione, carotenoids, and phenolic compounds) to protect plant cells (Bahmanbiglo et al., 2021; Abdallah et al., 2021; Mahmoud et al., 2022; El-Mogy et al., 2022; Pajoum et al., 2020; Abdelsattar et al., 2024). These results explained why the activity levels of SOD, POD, CAT, and APX and proline content increased in the leaves of tomato plants grown under alkaline stress conditions than ones grown in non-stressful conditions (Rady et al., 2020). The current study also showed that under alkalinity stress the activity level of studied enzymatic antioxidants and proline in treated plants with selenium were higher in untreated plants (Fig. 3B). These results indicated to the positive role of selenium in enhancement the proline concentration and the activity levels of antioxidant enzymes (Semida et al., 2021; Bahmanbiglo et al., 2021).

Several researchers confirmed that plants treated with low selenium concentration showed a greater proline accumulation, higher cell membrane stability index, and upper activity level of peroxidase and catalase enzymes, and lower malondialdehyde content in treated wheat plants than control (Xiaoqin et al., 2009). Mousavi et al. (2022) stated that in cucumber transplants under alkalinity stress and selenite nutrition, improving activity levels of enzymatic antioxidants was due to the protective role of selenium by reduction of ROS and modulation of osmotic pressure by osmolites such as proline.

Alkaline stress could be disturbed selective adsorption of  $K^+$ -Na<sup>+</sup> that leads to unbalance of K + -Na + in plant cells (Yang et al., 2007; Kumara et al., 2009), which increased the accumulation Na in plant tissues more than K, these findings were consisted with the results of this study. Whereas, the higher Na and Na/K ratio was observed in plants grown under alkalinity stress than plants grown in normal conditions (*Fig. 6A, C*). Furthermore, the exogenous application of selenium improved the accumulation of potassium in tomato plants in both conditions.

At the end, the application with selenium improves growth performance and the tolerance of alkalinity-stressed tomato plants by improving the photosynthesis pigments, activity level of antioxidant enzymes, proline accumulation, and the concentration of K in plant tissues.

#### Conclusions

According to the results of the present study, alkalinity stress damages the physiological behaviors of tomato plants. The application of selenium under alkaline stress conditions can efficiently diminish stress-induced damage. Whereas, exogenous

application of selenium improved plant biomasses, increased leaf chlorophyll content, enhanced the activity levels of antioxidant enzymes, upgraded proline accumulation, elevated the leaf relative water content, improved membrane stability index and potassium concentration in tissue of alkalinity-stressed plants. Meanwhile, it reduced sodium accumulation, MDA concentration, and the level of hydrogen peroxide. Therefore, these findings suggested that the application of selenium is conceded as a promising treatment that can be used for alleviating adverse impacts of alkalinity stress and improving the growth and productivity of tomato greenhouse.

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#### ELECTRONIC APPENDIX

This manuscript has an electronic appendix.