

# AMELORATION OF SALINITY STRESS IN TOMATO BY FOLIAR SPRAYS OF ASCORBIC ACID AND PROLINE

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## ABSTRACT

Globally, salinity stress ranks as one of the most detrimental abiotic stresses constraining agricultural performance. This study evaluated the efficacy of foliar-used ascorbic acid (AsA) and proline (Pro), used separately or concurrently, in mitigating the deleterious effects of saline irrigation (2000 and 4000 mg·L<sup>-1</sup>) on the growth and productivity of tomato (cv. Super Strain). Salinity stress significantly reduced plant height, leaf area, dry biomass as well as and yield components. It also led to marked declines in chlorophyll pigments (chl. *a*, *b*, and total chl.) and relative water content. In contrast, salinity stress induced increases in endogenous proline and total phenolic contents in leaves. Foliar use of AsA and Pro, whether used independently or in combination, successfully reduced the harmful consequences of salt stress, leading to notable improvements in growth traits, chlorophyll concentration, leaf water status, and fruit yield. Furthermore, treatments enhanced the activities of catalase, peroxidase, and superoxide dismutase and stimulated the endogenous production of proline and phenolic contents. The combined application of AsA and Pro consistently produced the most pronounced improvements across all measured parameters. These findings suggest that the integrated use of AsA and Pro as foliar sprays represents a potential strategy to improve salinity tolerance and sustain tomato productivity under saline conditions.

**Additional Keywords:** Antioxidant enzymes, chlorophyll, relative water content, vitamin C

## RESUMEN

### Atenuación del estrés salino en tomate mediante pulverizaciones foliares de ácido ascórbico y prolina

A nivel mundial, el estrés salino se clasifica como uno de los estreses abióticos más perjudiciales que limitan el rendimiento agrícola. Este estudio evaluó la eficacia del ácido ascórbico (AsA) y la prolina (Pro) aplicados por vía foliar, ya sea por separado o simultáneamente, para mitigar los efectos nocivos del riego salino (2000 y 4000 mg·L<sup>-1</sup>) en el crecimiento y la productividad del tomate (cv. Super Strain). El estrés salino redujo significativamente la altura de la planta, el área foliar, la biomasa seca y los componentes del rendimiento. También provocó una marcada disminución de los pigmentos de clorofila (clorofila *a*, *b* y clorofila total) y del contenido relativo de agua (CRH). Por el contrario, el estrés salino indujo aumentos en la prolina endógena y el contenido fenólico total en las hojas. El uso foliar de AsA y Pro, ya sea de forma independiente o en combinación, redujo con éxito las consecuencias negativas del estrés salino, lo que resultó en mejoras notables en las características de crecimiento, la concentración de clorofila, el estado hídrico de las hojas y el rendimiento de los frutos. Además, los tratamientos potenciaron la actividad de la catalasa, la peroxidasa y la superóxido dismutasa, y estimularon la producción endógena de prolina y contenido fenólico. La aplicación combinada de AsA y Pro produjo consistentemente las mejoras más pronunciadas en todos los parámetros medidos. Estos hallazgos sugieren que el uso integrado de AsA y Pro como pulverizaciones foliares representa una estrategia potencial para mejorar la tolerancia a la salinidad y mantener la productividad del tomate en condiciones salinas.

**Palabras clave adicionales:** Enzimas antioxidantes, clorofila, contenido relativo de agua, vitamina C

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## INTRODUCTION

Addressing global food security has become increasingly urgent due to rapid population growth

and escalating environmental pressures on agricultural systems (Ghanem *et al.*, 2024). Within these challenges, soil salinization is particularly detrimental, acting as a significant

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barrier to crop productivity by severely constraining plant growth and reducing yields (Chen *et al.*, 2022). In tomato plants (*Solanum lycopersicum*), salinity stress adversely affects productivity through disruptions in morphological, physiological, and biochemical processes (Sundararaman *et al.*, 2023). A primary consequence of salt stress is the decline in root water potential, leading to a condition termed physiological drought, which induces osmotic stress (Chourasia *et al.*, 2021). This osmotic disturbance, compounded by ion toxicity, contributes to the excessive buildup of reactive oxygen species (ROS), thereby leading to an imbalance in cellular redox homeostasis, membrane lipid peroxidation, and irreversible cellular damage that ultimately affects plant growth (Rahman *et al.*, 2023).

To counteract these detrimental effects, the external application of certain compounds has emerged as an effective approach to increase plant resilience under saline conditions (Yan *et al.*, 2021; El-Beltagi *et al.*, 2024). In particular, foliar applications of ascorbic acid and proline have demonstrated the ability to activate the salt overly sensitive signalling pathway, a key process for maintaining ion balance in saline environments (Meena *et al.*, 2019; Chen *et al.*, 2024). Ascorbic acid functions as a key antioxidant, playing a central role in redox regulation and defence responses by modulating gene expression and facilitating ROS detoxification (Ishikawa *et al.*, 2018). Serving both as an electron donor and antioxidant, AsA significantly contributes to plant development and environmental adaptability (Smirnoff *et al.*, 2018). It is localized in both the cytosol and the apoplast, where it helps sense environmental stimuli and regulate antioxidant and redox-responsive signalling pathways (Foyer *et al.*, 2011).

Proline, meanwhile, acts as a vital osmo-protectant under saline conditions by maintaining cellular osmotic balance and enhancing water-holding capacity (El Moukhtari *et al.*, 2020). Additionally, Pro enhances the activity of essential antioxidant enzymes *i.e.* superoxide dismutase (SOD), catalase (CAT), and ascorbate peroxidase (APX), thus reducing oxidative damage caused by ROS (Naz *et al.*, 2025). It also contributes to ionic homeostasis by limiting the absorption and transport of harmful ions such as

Na<sup>+</sup> and Cl<sup>-</sup>, while enhancing the uptake of beneficial ions like K<sup>+</sup> (El Moukhtari *et al.*, 2020). Moreover, proline supports photosynthetic efficiency by elevating chlorophyll levels and improving gas exchange, ultimately contributing to greater biomass and yield (Naz *et al.*, 2025).

While the separate application of ascorbic acid (AsA) and proline (Pro) has been demonstrated to optimize salinity tolerance in a range of plant species, their synergistic effects in tomato plants have not been thoroughly investigated. Consequently, this study seeks to investigate the efficacy of foliar-applied AsA and Pro, both individually and in combination, in reducing the harmful effects of salinity on tomato, with a particular focus on growth morphological, physiological, and biochemical traits.

## MATERIALS AND METHODS

Pot experiments were performed in two successive trials during the 2022 and 2023 seasons at the Department of Agricultural Botany, Kafrelsheikh University, Egypt, to assess the individual and combined effects of foliar-used ascorbic acid (2 mM) and proline (20 mg·L<sup>-1</sup>) on tomato plants. The treatments were applied under two salinity regimes of irrigation water (2000 and 4000 mg·L<sup>-1</sup>, corresponding to an electric conductivity values of 3.125 and 6.25 dS·m<sup>-1</sup>), obtained through seawater dilution, to evaluate their effects on vegetative and reproductive growth and selected physiological and biochemical attributes. The experiments were performed using clay loam soil, which was characterized according to the analytical procedures outlined by Chapman and Pratt (1978). Thorough details regarding the physical and chemical properties of the soil were described in El-Beltagi *et al.* (2024).

**Experimental design.** The experiment consisted of ten treatments in a split-plot design with four replications of ten plants per treatment, where salinity levels were assigned to the main plots and foliar applications of ascorbic acid and proline to the subplots. Tomato plants (*S. lycopersicum* cv. Super Strain B), obtained from the Legume Research Department, Field Crops Institute, Agricultural Research Center (Giza, Egypt), were utilized. In March, seeds were initially sown in peat moss under greenhouse

conditions to ensure uniform germination. Uniform seedlings were transplanted in April into polyethylene pots (30 cm in diameter  $\times$  40 cm in depth), following the specified treatment protocol. Pots were loaded with 8 kg of clay loam soil and had three drainage openings at the base to ensure proper drainage, fitted with sponges to regulate water outflow. Foliar applications of ascorbic acid and proline were performed twice: the first at 45 days after sowing and the second 15 days thereafter. Each pot received 100 mL of the designated treatment solution during each application.

**Fertilization.** Before sowing, P and K fertilizers were uniformly incorporated into the soil. Calcium superphosphate (15.5 %  $P_2O_5$ ) and potassium sulfate (48 %  $K_2O$ ) were each used at a rate of 1.8 g·pot<sup>-1</sup>. Nitrogen was provided as ammonium sulfate (20.5 % N), also at 1.8 g·pot<sup>-1</sup>, split into three uniform dosages administered at successive stages throughout the growing season.

**Samplings, measurements, and determinations.** For each treatment, a single sample of five plants per trait was randomly selected 70 days after transplanting in both growing seasons to assess the following variables.

**Growth characters.** The measured parameters comprised height of the plant (cm), dry weight/plant (g/plant), and leaf area (cm<sup>2</sup>/plant). Dry weight was determined by drying whole plant samples in a forced-air oven at 70 °C for 72 hours. Leaf area was measured using a portable laser leaf area meter (model CI-02; CID Bio-Science, USA).

**Relative Water Content (RWC).** It was determined based on the technique outlined by Kalapos (1994), and expressed in percentage.

**Chlorophyll pigments.** At 70 days after transplanting, the fourth fully expanded leaf from the apex of the main shoot was excised and immersed in 5 mL of dimethylformamide for pigment extraction. Chlorophyll *a*, *b*, and total chl. contents ( $\mu\text{g}\cdot\text{cm}^{-2}$ ) were quantified according to the method described by Moran (1982).

**Antioxidants enzymes.** At 70 days after planting in 2022 season, the third fully expanded leaf from the apex was collected and ground in liquid N for antioxidant enzyme assays. The frozen tissue was extracted using ice-cold 0.1 M Tris-HCl buffer (pH 7.5), supplemented with 5 % sucrose and 0.1 % 2-mercaptoethanol, at a ratio of three volumes of buffer to one of fresh tissue weight. The homogenate was centrifuged at

10,000  $\times$  g for 20 minutes at 4 °C, and the supernatant was collected for enzyme activity and protein assays. All extraction and assay steps were conducted at 4 °C to preserve enzymatic integrity and protect against degradation.

To assess antioxidant enzyme activities including catalase, peroxidase and superoxide dismutase, lipid peroxidation and total protein levels, 500 mg of fresh tomato leaf tissue was homogenized at -4 °C in 5 mL of 100 mM sodium phosphate buffer (pH 7.5) containing 1 mM ascorbic acid, 1 mM EDTA, 0.5 M NaCl, and 1 % Triton X-100. Centrifugation was performed on the homogenate at 20,000  $\times$  g (20 minutes at -4 °C) and the resulting supernatant was used for the quantification of enzyme activities, lipid peroxidation, and protein concentration.

Catalase (CAT; EC 1.11.1.6) activity was determined by measuring the rate of hydrogen peroxide ( $H_2O_2$ ) decomposition at 240 nm, following the method of Aebi (1984), and expressed as units per milligram of protein (U/mg protein), where one unit corresponds to the breakdown of 1  $\mu\text{mol}$  of  $H_2O_2$  per minute.

Peroxidase (POD; EC 1.11.1.7) activity was assessed using the procedure of Polle *et al.* (1994).

Superoxide dismutase (SOD; EC 1.15.1.1) activity was estimated according to the method of Giannopolitis and Ries (1977). One unit of SOD activity was defined as the amount of enzyme essential for inhibit 50 % of NBT reduction, with absorbance recorded at 560 nm. Superoxide dismutase activity was reported in USOD /mg of protein, equivalent to the  $\mu\text{M}$  of ascorbate oxidized/minute.

**Endogenous proline content and total phenols.** Fresh leaf samples were collected 70 days after sowing to quantify proline content, which was expressed in  $\mu\text{M}$ /gram of FW ( $\mu\text{mol}\cdot\text{g}^{-1}$  FW). The determination was carried out spectrophotometrically at 520 nm using a Shimadzu UV-1601 spectrophotometer, following the protocol as outlined by Bates *et al.* (1973). Additionally, total phenols content in leaf tissues was determined according to the protocol described by Bessada *et al.* (2016).

**Yield and yield quality.** During both growing seasons, fruits were harvested from five tomato plants between June 8 and June 14, and the total yield was recorded as grams per plant. Fresh, uniformly mature fruits from each treatment were

collected and instantly taken to the laboratory for analysis, where they were stored at  $-30^{\circ}\text{C}$  for subsequent quality assessments. The evaluated fruit quality parameters included total soluble solids (TSS), vitamin C content and titratable acidity (TA).

**TSS.** They were determined by placing fresh juice onto the glass prism of a digital pocket refractometer (QATAGO, Manual Brix 0.0-33.0 %, Japan), with values expressed in degrees Brix ( $^{\circ}\text{Brix}$ ) following standard procedures.

**TA.** It was measured using the technique established by Mohammadi *et al.* (2010). A total of 10 g of tomato pulp was blended with 20 mL distilled water using a kitchen blender (MX-799S, Panasonic), and the mixture was filtered through cotton wool. A 5 mL aliquot of the filtrate was titrated against 0.1 N sodium hydroxide following the addition of two drops of 1 % phenolphthalein indicator. The endpoint of titration was indicated by the emergence of a stable pink hue at pH 8.1, and titratable acidity was calculated as percent citric acid per 100 g of FW.

$\text{Titratable acidity (\%)} = \frac{\text{Titer vol. (ml)} \times \text{normality (0.1)} \times \text{vol. made up (20 ml)} \times 64\text{g (equivalent wt. of citric acid)}}{\text{wt. of sample for titration (5 ml)} \times 1000}$ .

**Statistical analysis.** All data were processed using Microsoft Excel 2016, and figures were generated with SigmaPlot 14.00. Analysis of variance was performed using CoStat software, and treatment means were compared using Duncan's multiple range test at the 0.05 significance level for a split plot design.

## RESULTS

**Plant height, leaf area and dry weight.** As shown in Figure 1, irrigation with saline water at concentrations of  $2000\text{ mg}\cdot\text{L}^{-1}$  (S1) and  $4000\text{ mg}\cdot\text{L}^{-1}$  (S2) led to significant decreases in plant height, leaf area, and DW of tomato. The magnitude of these reductions enhanced with higher salinity levels. However, the foliar use of AsA or Pro, either alone or concurrently, counteracted the adverse effects of salinity when compared to stressed-untreated plants. Notably, the combined use of AsA and Pro under both salinity levels effectively mitigated the negative impact on vegetative growth traits.

**Chlorophyll pigments.** As depicted in Figure 2, the data indicate that irrigation with saline

water at  $2000\text{ mg}\cdot\text{L}^{-1}$  (S1) and  $4000\text{ mg}\cdot\text{L}^{-1}$  (S2) significantly reduced chl. *a* and total chl. content in both growing seasons. Interestingly, S1 irrigation led to an increase in chl. *b*, whereas S2 caused a significant decline in chlorophyll *b* content across both seasons. Foliar application of AsA or Pro under saline conditions enhanced chlorophyll *a* level in both seasons. Regarding chlorophyll *b*, the foliar application of AsA or Pro significantly improved its content, particularly in the second season. The joint application of AsA and Pro via foliar spray under both salinity levels resulted in the highest accumulation of chlorophyll pigments, outperforming the treatments irrigated with saline water alone.

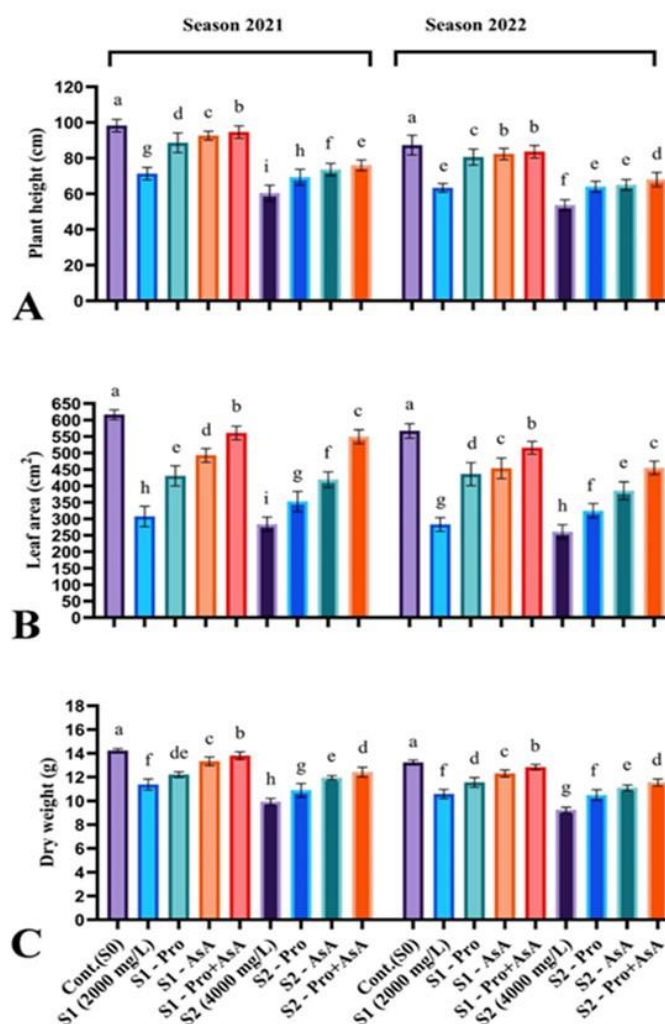
**Antioxidant enzymes.** The data presented in Figure 3 reveal that irrigation with saline water at both  $2000\text{ mg}\cdot\text{L}^{-1}$  and  $4000\text{ mg}\cdot\text{L}^{-1}$  significantly stimulated the activities of SOD, CAT as well as POD during the second growing season. Foliar application of either Pro or AsA further increased these enzymes activities under both salinity levels. Among the individual treatments, AsA proved more effective than Pro in enhancing antioxidant enzyme activity under salinity levels. Notably, foliar co-treatment with ASA and Pro induced the highest levels of CAT, POD, and SOD activities, indicating a synergistic effect under saline conditions.

**Endogenous proline, RWC and phenols content.** As shown in Figure 4, irrigation with saline water at  $2000\text{ mg}\cdot\text{L}^{-1}$  (S1) and  $4000\text{ mg}\cdot\text{L}^{-1}$  (S2) led to significant increases in leaf total phenolic content and endogenous proline in tomato during both seasons, except for proline in S2. Conversely, RWC was significantly reduced under both salinity levels. Foliar application of AsA or Pro notably enhanced, RWC, and under saline irrigation. Among the individual treatments, Pro was more effective than AsA in improving these parameters. The foliar co-application of AsA and Pro contributed to the highest increases in endogenous proline, RWC, and total phenolic compounds under both salinity levels across both seasons.

**Tomato fruit yield.** Tomato fruit yield per plant was significantly reduced under irrigation with diluted seawater at  $2000$  and  $4000\text{ mg}\cdot\text{L}^{-1}$  (Figure 5). However, foliar use of either Pro or AsA, individually induced a marked enhancement in fruit yield per plant under salinity stress relative

to untreated stressed plants. Among the individual treatments, AsA was more effective than Pro in enhancing yield under saline conditions. Notably, the concurrent use of Pro and AsA induced the

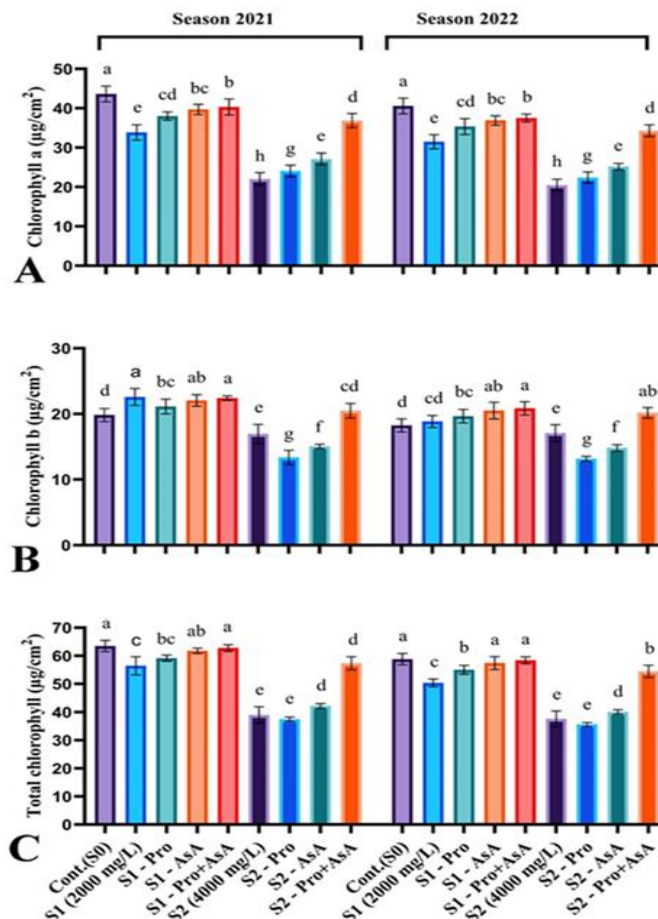
greatest improvement in fruit yield per plant under salinity stress, outperforming the individual treatments during both seasons.



**Figure 1.** Effects of foliar use of ascorbic acid, proline and their combination on tomato plant height (A), leaf area (B), and dry weight (C) under different salinity levels (2000 and 4000 mg·L<sup>-1</sup>), compared with the control treatment (freshwater irrigation; S0), during the 2021 and 2022 seasons. Distinct letters within each season means statistical differences among treatments according Duncan's multiple range test ( $p \leq 0.05$ ).

**Tomato fruit quality.** Fruit quality indicators *i.e.* titratable acidity (%) and vitamin C content were markedly decreased under 4000 mg·L<sup>-1</sup> saline irrigation (S2), as opposed to the control (Figure 6). Peak levels of vitamin C were recorded with the combined use of AsA and Pro under moderate salinity (2000 mg·L<sup>-1</sup>) during both

seasons. In contrast, TSS significantly increased under salinity stress in both years. The joint application via foliar spray of AsA and Pro resulted in the highest TSS values under high salinity conditions (4000 mg·L<sup>-1</sup>) across both seasons.



**Figure 2.** Effects of foliar application of ascorbic acid, proline and their combination on chlorophyll [chlorophyll a (A), b (B) and total chl. (C)] content under different salinity levels (2000 and 4000  $\text{mg}\cdot\text{L}^{-1}$ ), relative to the control (freshwater irrigation; S0), during the 2021 and 2022 seasons. Distinct letters within each season means statistical differences among treatments according Duncan's multiple range test ( $p\leq 0.05$ ).

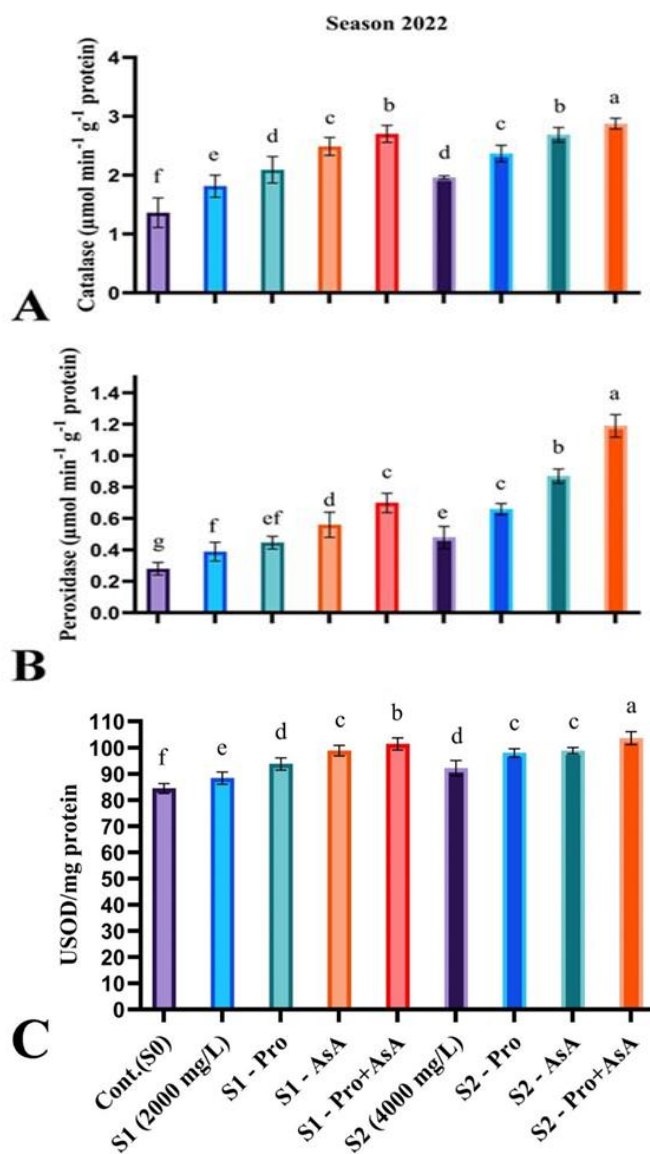
## DISCUSSION

The results of this study suggest a close relationship between the levels of salinity stress examined and the plant growth characteristics, including plant height and leaf area, as well as physiological traits such as RWC and chlorophyll content. Salinity represents a major abiotic stress that significantly constrain tomato (*S. lycopersicum*) yield and overall productivity, causing a wide range of morpho-physio-biochemical disturbances. High salt concentrations significantly reduce plant height, leaf area, and biomass by approximately 30 %, while also increasing the root-to-shoot ratio due to inhibited shoot growth (Azmat *et al.*, 2023). Despite adequate soil moisture, salinity

induces physiological drought by reducing water uptake and triggering the overproduction of ROS, which damage cellular structures, while salt-tolerant tomato genotypes typically exhibit enhanced antioxidant enzyme (SOD, CAT and POD) activity to counter oxidative stress (Guo *et al.*, 2022). Hormonal responses, including elevated levels of abscisic acid, ethylene, and salicylic acid, further regulate stress tolerance mechanisms. Reproductive development is also impaired, with salinity reducing fruit number, size, and total yield by more than 30 % at 200 mM NaCl though moderate salinity may augment distinct fruit quality traits (Guo *et al.*, 2022; Azmat *et al.*, 2023). Chlorophyll content (*a*, *b*, and total) is reduced due to ROS-induced chloroplast

damage, impaired pigment biosynthesis, and enhanced degradation (Al-Gasadi *et al.*, 2024), which ultimately limits photosynthesis. As a key adaptive response, proline accumulates in stressed tissues, where it serves as an osmo-protectant, stabilizes proteins and membranes, and scavenges

ROS. Enhanced proline accumulation is commonly observed in salt-tolerant cultivars relative to sensitive ones, highlighting its utility as a reliable biochemical marker for assessing plant responses to salinity stress (Gharsallah *et al.*, 2016; Al-Gasadi *et al.*, 2024).

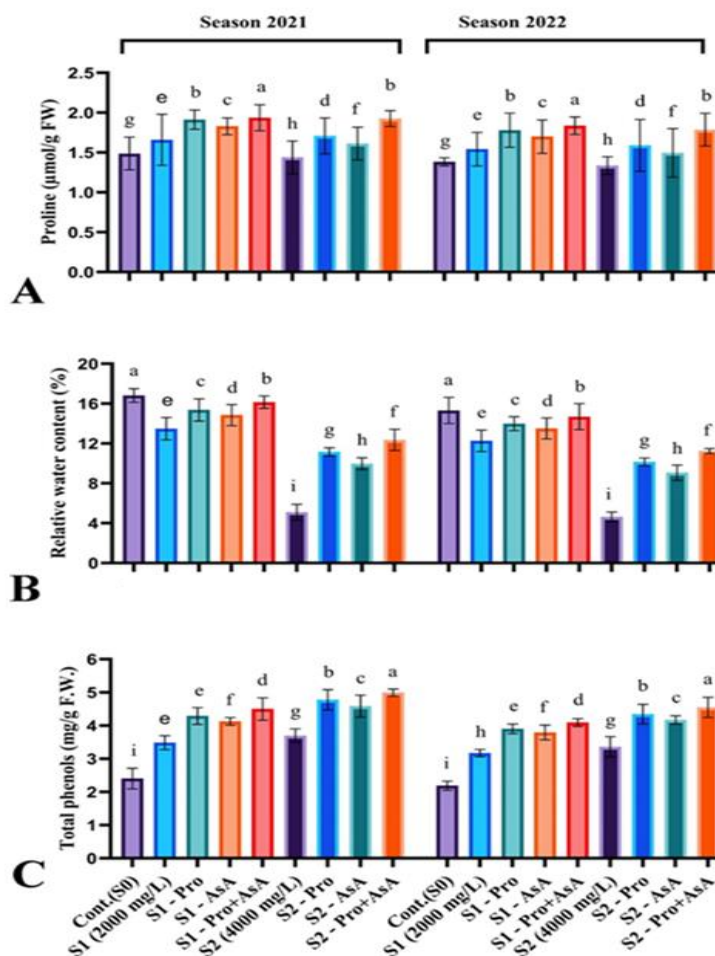


**Figure 3.** Effects of foliar use of ascorbic acid, proline and their combination on antioxidant enzymes activity; A: catalase, B: peroxidase, C: superoxide dismutase under different salinity levels (2000 and 4000  $\text{mg}\cdot\text{L}^{-1}$ ), compared with the control (freshwater irrigation; S0), during the 2022 season. Distinct letters within each season means statistical differences among treatments according Duncan's multiple range test ( $p \leq 0.05$ ).



The foliar applications of ascorbic acid effectively reduced the detrimental salinity stress effects in the tomato plants by enhancing both vegetative growth and physiological function. This indicates that ascorbic acid functions as a protective antioxidant, stabilizing chlorophyll and enhancing enzymatic defenses, total phenol content, and vitamin C levels, thereby alleviating the detrimental effects of salinity stress on tomato plants. Ascorbic acid promotes cell division and elongation, playing a role in increased shoot development even under saline conditions (Hossain *et al.*, 2017; Alnusaire *et al.*, 2022). It plays a pivotal role in maintaining redox homeostasis, facilitating cell wall expansion, and regulating enzyme activities essential for growth. Moreover, AsA preserves chlorophyll content by protecting chloroplast structures and stimulating

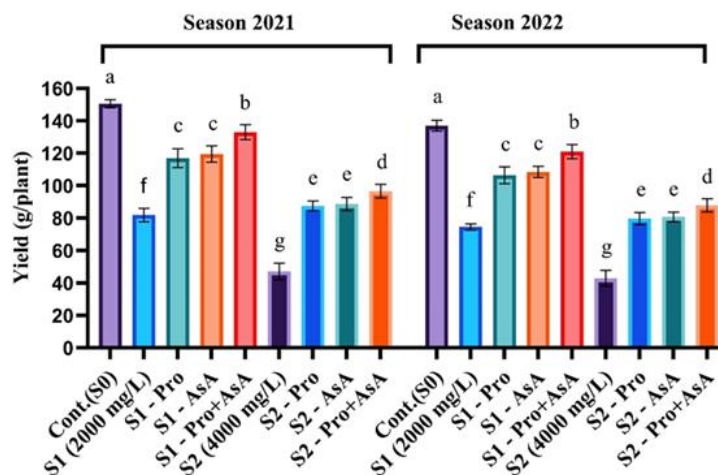
chlorophyll biosynthesis, thereby sustaining photosynthetic capacity (Xu *et al.*, 2018). It also enhances proline accumulation, supporting osmotic adjustment and stress tolerance, and induces the synthesis of phenolic compounds that strengthen the plant's antioxidant defense mechanisms (Xu *et al.*, 2018; Alnusaire *et al.*, 2022). Ascorbic acid application significantly increases the activities of SOD, catalase CAT and APX, which collectively mitigate oxidative damage and stabilize cellular structures (Hossain *et al.*, 2017; Xu *et al.*, 2018). In terms of productivity, AsA-treated plants exhibit higher fruit number, larger fruit size, and improved total yield under salinity stress, along with enhanced fruit quality traits such as elevated sugar content, firmness, and nutritional compounds including vitamin C and lycopene (Xu *et al.*, 2018).



**Figure 4.** Impact of foliar-applied ascorbic acid, proline and their combination on A: proline content ( $\mu\text{mol}\cdot\text{g}^{-1}\text{FW}$ ), B: RWC (%), and C: total phenolic content ( $\text{mg}\cdot\text{g}^{-1}\text{FW}$ ) in tomato plants under



salinity stress conditions (2000 and 4000 mg·L<sup>-1</sup>), compared with the control (freshwater irrigation; S0), during the 2021 and 2022 growing seasons. Distinct letters within each season means statistical differences among treatments according Duncan's multiple range test ( $p \leq 0.05$ ).



**Figure 5.** Impact of foliar-applied ascorbic acid, proline and their combination on tomato fruit yield under salinity stress conditions (2000 and 4000 mg·L<sup>-1</sup>), compared with the control (freshwater irrigation; S0), during the 2021 and 2022 growing seasons. Distinct letters within each season means statistical differences among treatments according Duncan's multiple range test ( $p \leq 0.05$ ).

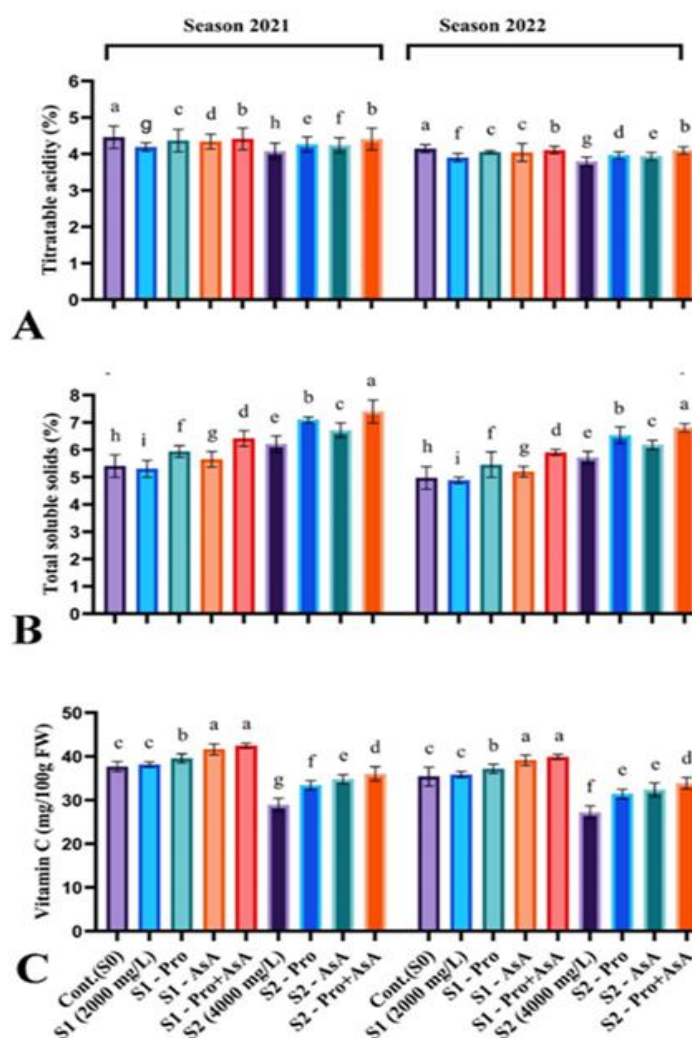
A correlation was observed between endogenous proline content of the tomato plants and various morphological, physiological, and biochemical traits, including plant growth characteristics and antioxidant enzyme activities. The foliar application of proline effectively mitigated the salinity stress adverse effects on the plants by enhancing physiological performance, biochemical responses, and yield-related traits. Proline contributes to improved water retention and cell turgor, which supports vegetative growth, including increased plant height, leaf area, and DW under salinity stress conditions (Shafi *et al.*, 2019; Mundada *et al.*, 2021). Functioning as a key osmo-protectant, proline stabilizes cellular membranes and proteins while maintaining intracellular hydration. Its application also helps preserve chlorophyll content by protecting chloroplasts from oxidative damage and limiting ROS-induced pigment degradation. Exogenous proline enhances endogenous proline accumulation, further improving osmotic adjustment and stress tolerance (Mundada *et al.*, 2021). Additionally, proline promotes the synthesis of phenolic compounds, which play

crucial roles in scavenging ROS and enhancing the plant's antioxidant defense system (Shafi *et al.*, 2019). Proline application significantly increases the activity SOD, CAT and POD, aiding in ROS detoxification and protection of cellular structures (Mundada *et al.*, 2021; Singh *et al.*, 2022). These physiological and biochemical enhancements translate into improved fruit set, fruit size, and overall yield, as well as improved fruit quality traits such as firmness, and nutritional value, including elevated levels of vitamin C and lycopene (Mundada *et al.*, 2021).

Foliar treatment with a combination of AsA and Pro demonstrated to significantly alleviate the harmful consequences of saline conditions in tomato by synergistically enhancing physiological, biochemical, and yield-related traits. This integrative approach improves salt tolerance by promoting cell division, water retention, and nutrient uptake, thereby enhancing vegetative growth under saline conditions (Kaur *et al.*, 2023). Ascorbic acid contributes to redox homeostasis and cell wall expansion, while proline stabilizes cellular membranes and proteins. Their combined application effectively preserves

chlorophyll content by protecting chloroplast structures, enhancing chlorophyll biosynthesis, and reducing degradation caused by ROS (Kaur *et al.*, 2023). Exogenous proline elevates internal proline levels, facilitating osmotic adjustment, whereas AsA supports proline metabolism and modulates stress-responsive gene expression (Alnusaire *et al.*, 2022). Furthermore, the combined treatment stimulates the synthesis of phenolic compounds, which serve as potent antioxidants and strengthen the plant's oxidative stress defense mechanisms (Kaur *et al.*, 2023). Activities of key antioxidant enzymes are also

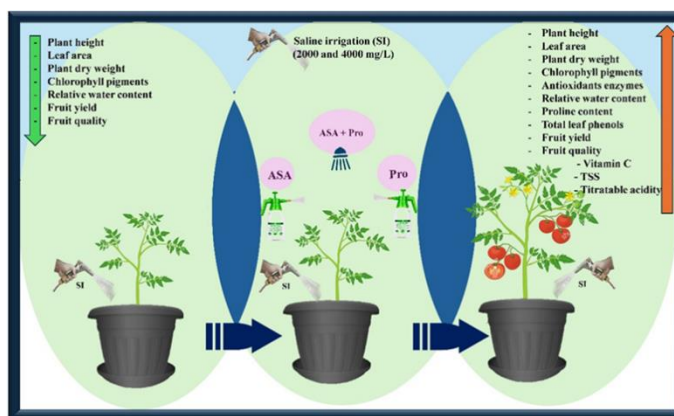
significantly enhanced, thereby minimizing ROS accumulation and preserving cellular integrity (Alnusaire *et al.*, 2022; Kaur *et al.*, 2023). The results revealed that the combined application of ascorbic acid and proline enhanced plant growth traits, chlorophyll content, endogenous proline levels, and antioxidant enzyme activities. These improvements were closely associated with increased fruit yield and quality, suggesting that this synergistic treatment is a promising strategy to boost tomato resilience and productivity under saline conditions.



**Figure 6.** Impact of foliar-applied ascorbic acid, proline and their combination on tomato fruit quality including titratable acidity % (A), total soluble solid (B) and vitamin C (C) under salinity stress conditions (2000 and 4000 mg·L<sup>-1</sup>), when contrasted with the control treatment (freshwater irrigation), during the 2021 and 2022 growing seasons. Distinct letters within each season means statistical differences among treatments according Duncan's multiple range test ( $p \leq 0.05$ ).

Figure 7 shows the negative impact of saline irrigation on the growth of tomato plants, adversely affecting growth and yield attributes, antioxidant enzyme activities, RWC and total

phenols. However, foliar application of ascorbic acid and proline, either alone or in synergy, effectively counteracted these adverse effects in all assessed traits.



**Figure 7.** Illustration of the harmful effects of saline irrigation (SI) (2000 or 4000 mg·L<sup>-1</sup>) on tomato plants, including impacts on growth, physiological, and biochemical parameters. The figure also reveals the mitigation of these adverse effects through foliar application of ascorbic acid (2 mM) and proline (20 mg·L<sup>-1</sup>), applied either individually or in combination.

## CONCLUSION

The application of ascorbic acid and proline, alone or in combination, improves tomato tolerance to salinity stress by enhancing growth traits, chlorophyll content, phenolic compounds, relative water content, endogenous proline, and antioxidant enzyme activities, ultimately increasing fruit yield and quality, including titratable acidity, total soluble solids and vitamin C content. The combined application produced the most pronounced improvements across all traits studied.

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