Revista de
CIENCIAS AGRÍCOLAS

e-ISSN Online 2256-2273

Received: June 5 2025 Accepted: November 28 2025 Published: December 22 2025

Research article: Plant science

Impacts of three ascorbic acid application methods on the growth of salt-affected tomato seedlings

Impacto de tres métodos de aplicación de ácido ascórbico en el crecimiento de plántulas de tomate bajo estrés salino

Faouzi Horchani [1]; Amal Bouallegue [2]; Arij Bouazzi [3]; Zouhaier Abbes [4]

[1] *University of Gafsa, Gafsa, Tunisia, faouzih20056@yahoo.fr, https://orcid.org/0000-0003-1322-978X (Correspondence)*
[2] *University of Carthage, Tunis, Tunisia, amal.bouallegue@hotmail.fr, https://orcid.org/0000-0001-5632-2373*
[3] *University of Carthage, Tunis, Tunisia, bouazziarij89@gmail.com, https://orcid.org/0009-0005-9225-4263*
[4] *University of Carthage, Tunis, Tunisia, zouhaier_abbes@yahoo.fr, https://orcid.org/0000-0003-4419-4639*

Cite: Horchani, F.; Bouallegue, A.; Bouazzi, A.; Abbes, Z.(2025). Impacts of three ascorbic acid application methods on the growth of salt-affected tomato seedlings. *Revista de Ciencias Agrícolas*. 42(3): e3276. https://doi.org/10.22267/rcia.20254203.276

ABSTRACT

Ascorbic acid (AsA) is a well-known antioxidant that improves plant tolerance to salt stress; however, its effectiveness has not yet been sufficiently demonstrated depending on the method of application. In this context, this study aimed to investigate the effects of 1 mM AsA applied as a priming agent (AsA/P), through the rooting medium (AsA/R), or via foliar spraying (AsA/F) on the growth, key biochemical parameters, and the antioxidant defense system of tomato seedlings (cv. Rio Grande) grown for two weeks in the presence of 100 mM NaCl. Results showed a noticeable reduction in growth traits, with significant decreases in relative water content (RWC), chlorophyll, total carbohydrates, proline, polyphenols, and AsA content, as well as in the activities of superoxide dismutase (SOD), catalase (CAT), and ascorbate peroxidase (APX). However, the contents of hydrogen peroxide (H_2O_2) and malondialdehyde (MDA) were significantly increased in salt-treated compared to control seedlings. Regardless of the application method, AsA improved seedling growth and increased RWC, chlorophyll, total carbohydrates, and proline contents. Furthermore, the contents of AsA and polyphenols, as well as the activities of SOD, CAT, and APX, were significantly enhanced, leading to a substantial decrease in the contents of H_2O_2 and MDA. The results also indicated that the AsA-induced mitigating effects were more prominent with the AsA/R method, followed by the AsA/F, whereas the AsA/P method was less effective. In conclusion, compared to the AsA/F and AsA/P methods, the AsA/R treatment can be considered an efficient and promising method to ameliorate the growth of tomato seedlings in salt-affected soils.

Keywords: Antioxidant; ascorbic acid; exogenous application; oxidative stress; salinity; *Solanum lycopersicum* L.

RESUMEN

El ácido ascórbico (AsA) es un reconocido antioxidante que mejora la tolerancia de las plantas al estrés salino; sin embargo, la influencia del método de aplicación sobre su eficacia aún no ha sido evaluada de manera exhaustiva. En este contexto, el presente estudio se llevó a cabo con el objetivo de analizar los efectos de 1 mM de AsA aplicado como agente de cebado (AsA/P), a través del medio de enraizamiento (AsA/R) o mediante pulverización foliar (AsA/F), sobre el crecimiento, algunos parámetros bioquímicos clave y el sistema de defensa antioxidante en plántulas de tomate (cv. Río Grande) se cultivó durante dos semanas bajo condiciones de 100 mM de NaCl. Los resultados obtenidos evidenciaron una reducción significativa en las características de crecimiento, asociada con disminuciones notables en el contenido relativo de agua (RWC), clorofila, carbohidratos totales, prolina, polifenoles y AsA, así como en las actividades de superóxido dismutasa (SOD), catalasa (CAT) y ascorbato peroxidasa (APX). En contraste, los niveles de peróxido de hidrógeno (H_2O_2) y malondialdehído (MDA) aumentaron drásticamente en las plántulas sometidas a salinidad en comparación con el control. Independientemente del método de aplicación, el AsA promovió el crecimiento de las plántulas y elevó los contenidos de RWC, clorofila, carbohidratos totales y prolina. Asimismo, los niveles de AsA y polifenoles, junto con las actividades enzimáticas de SOD, CAT y APX, se incrementaron de forma significativa, lo que se tradujo en una reducción considerable de H_2O_2 y MDA. Los hallazgos también mostraron que los efectos mitigadores del AsA fueron más evidentes cuando se aplicó mediante el medio de enraizamiento (AsA/R), seguidos por la pulverización foliar (AsA/F), mientras que el cebado de semillas (AsA/P) resultó menos eficaz. En conclusión, en comparación con los métodos AsA/F y AsA/P, la aplicación de AsA a través del enraizamiento (AsA/R) puede considerarse una estrategia eficiente y prometedora para favorecer el crecimiento de plántulas de tomate en suelos afectados por salinidad.

Palabras clave: Antioxidante; ácido ascórbico; aplicación exógena; estrés oxidativo; salinidad; *Solanum lycopersicum* L.

INTRODUCTION

Under both natural and agricultural conditions, plants are frequently exposed to abiotic stresses (Zhao *et al.*, 2021). Among these, salt stress is gaining attention due to its adverse effect on agricultural production, resulting in a significant reduction in crop yield and, consequently, global food insecurity (El-Sabagh *et al.*, 2021). As one of the main limiting factors for plant growth and development, salt stress can hinder plant respiration, root water absorption, mineral uptake and assimilation, stomatal movement, photosynthesis, enzyme activities, and hormone balance (Arif *et al.*, 2020; Aazami *et al.*, 2021). In addition to these primary effects, a secondary oxidative stress can occur due to the excessive accumulation of reactive oxygen species (ROS) and the disruption of the balance between the generation of ROS and antioxidant defense systems, leading to severe damage in the structure and the functioning of major plant cells (Arif*et al.*, 2020; Xu *et al.*, 2022).

Tomato is one of the most important crops, offering significant economic and nutritional values. In addition to being a major source of dietary fiber, tomato provides considerable amounts of vitamins (A and C) and carotenoids (mainly lycopene), as well as micro and macronutrients, which are beneficial for human health (Liu *et al.*, 2018). However, like many other crop plants, tomato production does not meet requirements, as it is frequently exposed to multiple abiotic constraints, mainly soil salinity. Regarding the severe negative effects of salt stress on its growth and productivity, tomato is considered sensitive to this constraint (Guo *et al.*, 2022). Furthermore, due to the insufficient rainfall, the use of saline water for crop irrigation has become necessary, particularly in semi-arid and arid regions (Jha *et al.*, 2019; Zhao *et al.*, 2021). The use of exogenous plant growth regulators (PGRs) to enhance growth and increase productivity in many plant species under saline conditions has received considerable attention from scientists. Among these PGRs, ascorbic acid (AsA) stands out, an essential antioxidant widely used in agriculture due to its effectiveness in the mitigation of the devastating effects of salt stress (Chen *et al.*, 2023). It has been shown that AsA plays a crucial role in cell division and elongation, osmoregulation, and hormone biosynthesis, all of which are necessary processes for plant growth and development (Hassan *et al.,* 2021). AsA is involved in regulating the transpiration and the movements of stomata, protecting photosynthesis structures and photosynthetic pigments, as well as regulating both ionic and osmotic balance within plant organs (Billah *et al.*, 2017; Chen *et al.*, 2023). Furthermore, AsA constitutes an essential line of plant defense against the salt-induced oxidative stress by removing many types of free radicals, primarily as a substrate of ascorbate peroxidase, a vital enzyme in the ascorbate/glutathione pathway (Hassan *et al.*, 2021). Several studies pointed to a crucial role of AsA in activating several ROS-scavenging enzymes, such as superoxide dismutase, catalase, and peroxidases, all of which are involved in protecting macromolecules against salt-induced oxidative damage (Chen *et al.*, 2023; Kanwal *et al.*, 2024).

As described above, several previous studies showed the ability of the exogenously-applied AsA to ameliorate plant tolerance to salt stress (Mittal *et al.*, 2018; Chen *et al.*, 2023; Ajila-Celi *et al.*, 2025). However, the AsA-mediated tolerance depends upon various aspects, such as the severity of salt stress, species type, plant growth stage, as well as the applied dose and the manner of application of this growth regulator (Njus *et al.*, 2020). Although AsA may be applied to plants through seed priming, root application, or foliar spraying, these methods in tomato under salinity remains poorly documented in the literature. Therefore, the present study was conducted to compare the effectiveness of seed priming, root application, and foliar spraying of AsA on some growth attributes of tomato seedlings under saline conditions, and to determine which method of AsA application is more efficient and suitable for tomato cultivation in salt-affected fields.

MATERIAL AND METHODS

Plant material, growth conditions, and experimental treatments

Tomato seeds (*Solanum lycopersicum* L., cv. Rio Grande) were sterilized in a 10% sodium hypochlorite solution for 15 min and then washed three times with sterile distilled water. Thereafter, seeds were soaked for 12h for priming in 1 mM ascorbic acid (AsA) and then rinsed thoroughly with distilled water. Primed seeds, along with unprimed seeds, were germinated in Petri dishes in the dark at 23 °C for one week. Seedlings obtained from AsA-primed (denoted as AsA/P) and unprimed seeds were grown in a loamy mixture with clean sand soil (1/1, w/w) in a glasshouse under controlled conditions (28 °C/20 °C day/night temperature, 70-75% relative humidity, and a 16 h photoperiod). Salt treatment (100 mM NaCl) was applied three days after transplantation. At this moment, AsA treatments were also applied for seedlings derived from unprimed seeds. Three sets of treatments were implemented depending on the concentration and the manner of AsA application. In the first set, seedlings were not treated with AsA (denoted as S). In the second set, 1 mM AsA was applied to tomato seedlings through the rooting medium (denoted as AsA/R). In the third set, seedlings were foliar sprayed with 1 mM AsA (denoted as AsA/F). Seedlings obtained from unprimed seeds and not treated with NaCl were set as controls (denoted as C). Treatment conditions followed in the present study are shown in Table 1. The concentrations of 100 mM NaCl-salinity and 1 mM AsA used in this work were selected based on previous published experiments (Manaa *et al.*, 2014; Zarai *et al.*, 2022; Horchani *et al.*, 2024).

Table 1. *Different treatments used in the present study and their denotation.*

Treatment	Denotation
Control (without NaCl and AsA treatments)	C
100 mM NaCl-salinity (without AsA treatment)	S
Priming with 1 mM AsA (with100 mM NaCl treatment)	S +AsA/P
Root application of 1 mM AsA (with100 mM NaCl treatment)	S +AsA/R
Foliar spraying of 1 mMAsA (with100 mM NaCl treatment)	S +AsA/F

Growth parameters and water status analysis

Shoot and root fresh weights (FWs) were measured immediately after two weeks of treatments. For the determination of turgor weights (TWs), roots and shoots were immersed in distilled water for 4h and then weighed. Dry weights (DWs) were obtained by weighing the dried plant material. DW production was calculated as: DW_{f} _ DW_{o}, where DW_{f} and DW_{o} represent the DW obtained at the end of the experiment and just before the application of treatments, respectively. Leaf area (LA) was determined as described by Horchani *et al.* (2008). Briefly, the leaf was first placed on a sheet of paper, its area drawn, and the corresponding paper surface cut and weighed. LA value was then deduced from the weight of a 1 cm^2 piece of the paper. Leaf mass per area (LMA) was calculated as DW/LA ratio (Horchani *et al.*, 2008). Relative water content (RWC) was calculated as (FW-DW)/ (TW-DW) *100 (Silveira *et al.*, 2003).

Total chlorophyll, soluble carbohydrates, and proline content determination

Total chlorophyll contents were determined in freshly harvested leaves according to the method of Arnon *et al.* (1949). Total soluble carbohydrate and proline contents were estimated using the anthrone-sulphuric acid (McCready *et al.*, 1950) and ninhydrin (Bates *et al.*, 1973) methods, respectively.

Hydrogen peroxide and malondialdehyde content determination

Hydrogen peroxide (H_2O_2) content was determined using the potassium iodide method as described by Junglee *et al.* (2014). Lipid peroxidation, estimated as malondialdehyde (MDA) content, was measured following the reaction of thiobarbituric acid, as reported by Fu and Huang (2001).

Total polyphenols and ascorbate determination

Total polyphenol contents were determined using the Folin-Ciocalteu reagent method (Sarker & Oba, 2020) using gallic acid as a standard. Ascorbate was assayed based on the reduction of Fe^{3+} to Fe^{2+} by ascorbate as described by Nazar*et al.* (2015).

Antioxidant enzyme activities determination

Superoxide dismutase (SOD), catalase (CAT), and ascorbate peroxidase (APX) were extracted as described by Horchani *et al.* (2024). Briefly, freshly-harvested root and leaf samples were homogenized under ice-cold conditions in 50 mM potassium phosphate buffer (pH 7.8) added with 10% PVP, 1 mM PMSF, 2 mM EDTA, 10 mM DTT, and 0.1% Triton X- 100. Thereafter, the homogenate was centrifuged at 10.000

g for 20 min at 4°C, and the obtained supernatants were used for enzymes assay.

SOD activity was assayed by measuring the inhibition of the photochemical reduction of nitroblue tetrazolium by the enzyme according to the method of Giannopolitis and Ries (1977). CAT activity was estimated by following the decomposition of H_2O_2 as described by Hasanuzzaman *et al*. (2023). APX activity was measured according to Nazar *et al*. (2015) by recording the decrease in absorbance of ascorbate at 290 nm and was calculated by using the extinction coefficient 2.8 mM^{-1} cm^{-1}. One unit of enzyme corresponds to the amount necessary to decompose 1 μmol of substrate per minute at 25 °C.

Data analysis and statistics

Analysis of variance (ANOVA) was performed to assess the differences among treatments. The analysis consisted of a one-way model with treatment (seven levels, random factor, six replicates). The XLSTAT 2023 software was used. Experiments were replicated two to three times. Differences at a level of $p \leq 0.05$ were considered significant.

RESULTS

Effects of AsA treatments on the growth of salt-stressed tomato seedlings

In order to review if AsA can be involved in the amelioration of the growth of tomato seedlings under non-stressful conditions, preliminary experiments were performed. Tomato seedlings derived from AsA-primed seeds or treated with 1 mM AsA (through the rooting medium or foliar spraying) were cultivated in the absence of NaCl and analyzed after two weeks for their DW production, RWC, LA, and LMA. The results indicated no obvious effects of AsA treatments on all analyzed growth parameters (data not shown). These results indicate that the level of endogenously-synthesized AsA is sufficient to regulate, under control conditions, the different physio-biochemical pathways in which this antioxidant is involved. For simplicity, only the data obtained under saline conditions will be presented in the present study.

As shown in Table 2, salt-stressed tomato seedlings exhibited a decrease in all measured growth parameters. Shoot dry weight production (SDW), root dry weight production (RDW), shoot relative water content (SRWC), and root relative water content (RRWC) were reduced by 44, 50, 23, and 26%, respectively, as compared to controls. LA and LMA were significantly decreased and increased, respectively, by 40% and 54% relative to controls.

Although all AsA-treatments noticeably increased DW production, LA, and RWC of salt-stressed tomato seedlings, the highest levels of increase were observed following the application of AsA through the rooting medium (AsA/R treatment), followed by the foliar spray method (AsA/F). The lowest levels of increase were recorded for plants obtained from AsA-primed seeds (AsA/P). Compared to salt-stressed seedlings untreated with AsA, SDW, RDW, SRWC, RRWC, and LA were increased by 55, 75, 28, 26, and 67%, as well as by 35, 50, 21, 17, and 38%, following the AsA/R and AsA/F treatments, respectively. Increases of 20, 25, 10, 11, and 19% were observed in SDW, RDW, SRWC, RRWC, and LA, respectively, following the AsA/P treatment. LMA was decreased by 25 and 16%, following the AsA/R and AsA/F treatments, respectively, whereas no obvious effect was observed for the AsA/P treatment (Table 2).

Table 2. *DW and RWC of shoots and roots, LA and LMA of Solanum lycopersicum cv. Rio Grande seedlings grown for 2 weeks under 100 mM NaCl-salinity and 1 mM AsA.*

	Shoot DW (g plant^{-1})	Root DW (g plant^{-1})	Shoot RWC (%)	Root RWC (%)	LA (cm^2 plant^{-1})	LMA (g m^{-2})
C	0.36 ± 0.03^a	0.24 ± 0.02^a	82.5 ± 1.5^a	75.2 ± 1.0^a	25.50 ± 1.50^a	1.85 ± 0.13^e
S	0.20 ± 0.02^e	0.12 ± 0.01^e	60.4 ± 2.2^e	55.8 ± 2.0^e	13.61 ± 1.32^e	2.85 ± 0.08^a
S+AsA/P	0.24 ± 0.01^d	0.15 ± 0.00^d	66.7 ± 2.0^d	61.8 ± 1.7^d	16.23 ± 1.04^d	2.75 ± 0.05^a
S+AsA/R	0.31 ± 0.01^b	0.21 ± 0.01^b	77.5 ± 1.2^b	70.1 ± 1.6^b	22.71 ± 0.85^b	2.15 ± 0.10^d
S+AsA/F	0.27 ± 0.01^c	0.18 ± 0.01^c	73.2 ± 1.4^c	65.5 ± 1.2^c	18.85 ± 1.00^c	2.40 ± 0.04^c

Treatments were: (1**) control (C, without NaCl and AsA treatments), (**2**) salinity (S, treatment with 100 mM NaCl), (**3**) salinity + AsA applied through seed priming (S + AsA/P), (**4**) salinity + AsA applied through the rooting medium (S + AsA/R), and (**5**) salinity + AsA applied through foliar spraying (S + AsA/F). Values are the means of six replicates ± S.D. Within rows, significantly different values are followed by different letters according to the one-way ANOVA test at $p < 0.05$.

Effects of AsA treatments on the leaf chlorophyll contents of salt-stressed tomato seedlings

The results of the present study indicated that salt stress significantly decreased total leaf chlorophyll content by 32%, as compared to the control seedlings (Fig. 1), indicating the sensitivity of the Rio Grande tomato cultivar to salt stress. Regardless of its method of application, AsA significantly increased the total chlorophyll content of salt-stressed tomato seedlings (Fig. 1). Indeed, total chlorophyll content was increased by 14% for seedlings derived from AsA-primed seeds, whereas a greater increase of almost 30% was obtained following the AsA/R and AsA/F treatments (Fig. 1).

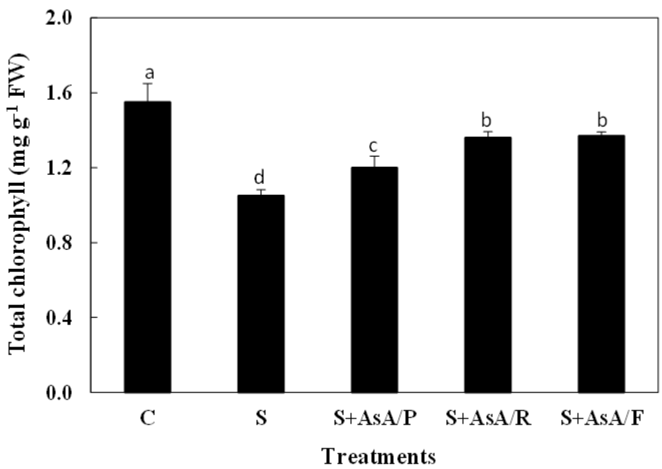
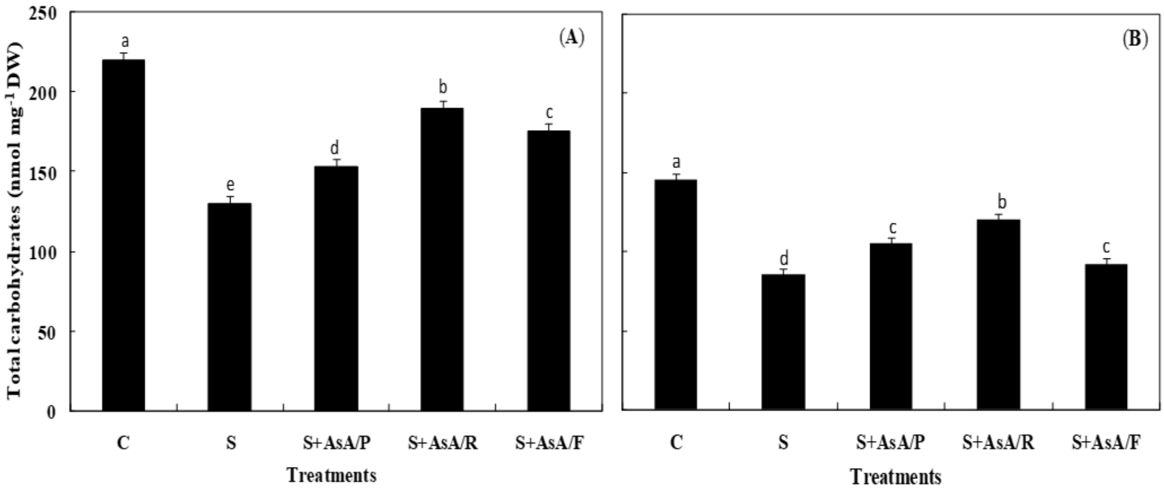


Figure 1. *Total chlorophyll content in leaves of Solanum lycopersicum cv. Rio Grande seedlings grown for two weeks under 100 mM NaCl-salinity and 1 mM ascorbic acid (AsA).*

*Treatments were: (1) control (C, without NaCl and AsA treatments), (2) salinity (S, treatment with 100 mM NaCl), (3) salinity + AsA applied through seed priming (S + AsA/P), (4) salinity + AsA applied through the rooting medium (S + AsA/R), and (5) salinity + AsA applied through foliar spraying (S + AsA/F). Values are the means of six replicates ± S.D. Significantly different values are followed by different letters according to the one-way ANOVA test at $p < 0.05$.

Effects of AsA treatments on the total carbohydrate contents of salt-stressed tomato seedlings

As shown in Figure 2, exposure to salt stress significantly decreased total carbohydrate contents by approximately 42% in roots and leaves of tomato seedlings. Results regarding the effects of AsA showed an increase in the carbohydrate contents following the exogenous application of this signal molecule. Nevertheless, the levels of increase depended on the mode of AsA supplementation. Indeed, with the AsA/R method, total carbohydrate contents were significantly increased by 46% and 41% in leaves and roots, respectively, as compared to seedlings treated only with NaCl; whereas, an increase of only 18 and 23% was observed in leaves and roots, respectively, when AsA was applied through the seed priming method. Although AsA-foliar spraying significantly increased total carbohydrate contents in leaves by approximately 35%, it did not affect those of roots (Figure 2).

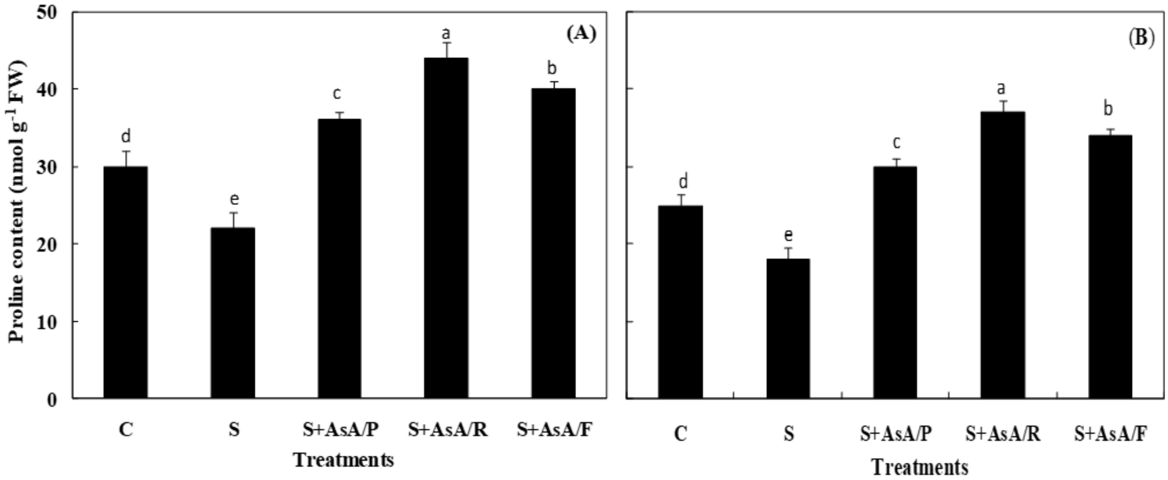


*Treatments were: (1) control (C, without NaCl and AsA treatments), (2) salinity (S, treatment with 100 mM NaCl), (3) salinity + AsA applied through seed priming (S + AsA/P), (4) salinity + AsA applied through the rooting medium (S + AsA/R), and (5) salinity + AsA applied through foliar spraying (S + AsA/F). Values are the means of six replicates ± S.D. Significantly different values are followed by different letters according to the one-way ANOVA test at $p< 0.05$.

Figure 2. *Total carbohydrate contents in leaves (A) and roots (B) of Solanum lycopersicum cv. Rio Grande seedlings grown for two weeks under 100 mM NaCl-salinity and 1 mM ascorbic acid (AsA).*

Effects of AsA treatments on the proline contents of salt-stressed tomato seedlings

The results of the current study showed that salt stress significantly reduced proline contents by 28% in leaves and roots, as compared to the control seedlings (Figure 3). Conversely, regardless of its method of application, AsA noticeably increased the proline contents of salt-stressed tomato seedlings (Figure 3). The highest levels of increase were achieved as a result of AsA/R treatment (increases of 100% and 106% in leaves and roots, respectively), followed by AsA/F treatment (increases of 82% and 89% in leaves and roots, respectively); whereas, the lowest levels of increase of proline contents were obtained following the AsA/P treatment (increases of 63% and 67% in leaves and roots, respectively) (Figure3).

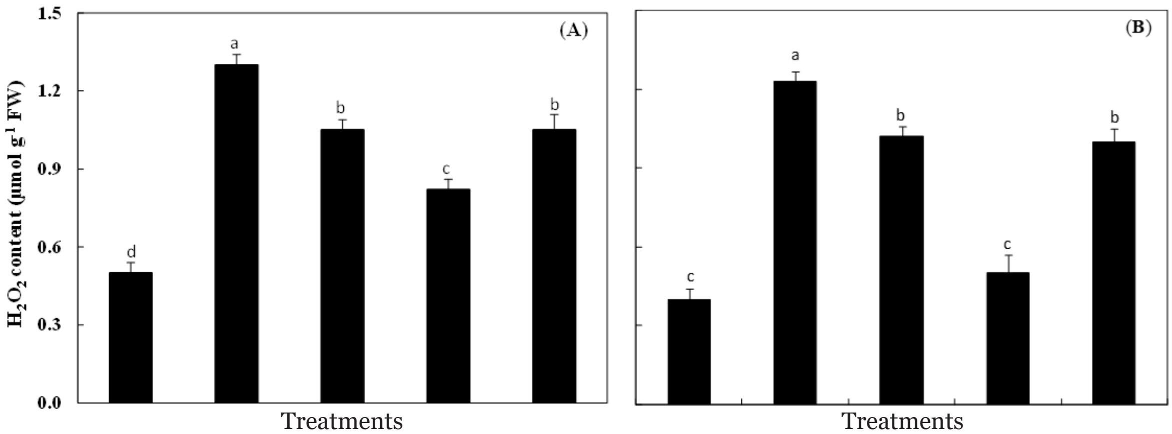


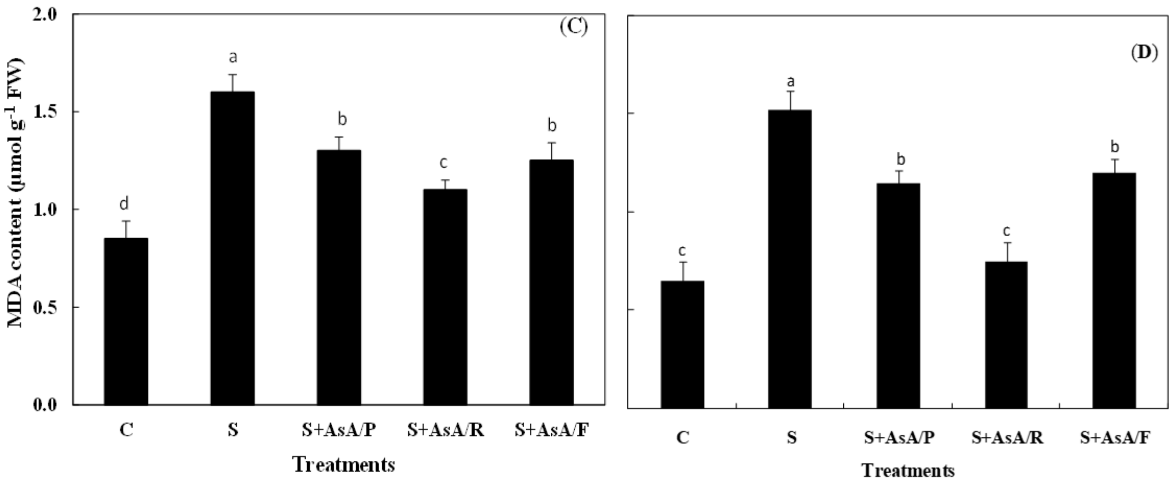
*Treatments were: (1) control (C, without NaCl and AsA treatments), (2) salinity (S, treatment with 100 mM NaCl), (3) salinity + AsA applied through seed priming (S + AsA/P), (4) salinity + AsA applied through the rooting medium (S + AsA/R), and (5) salinity + AsA applied through foliar spraying (S + AsA/F). Values are the means of six replicates ± S.D. Significantly different values are followed by different letters according to the one-way ANOVA test at $p< 0.05$.

Figure 3. *Proline contents in leaves (A) and roots (B)of Solanum lycopersicum cv. Rio Grande seedlings grown for two weeks under 100 mM NaCl-salinity and 1 mM ascorbic acid (AsA).*

Effects of AsA treatments on the oxidative damage marker contents of salt-stressed tomato seedlings

Salt stress induced oxidative damage in tomato seedlings, as indicated by the significant increases in H_2O_2 and MDA contents (Figure4). The H_2O_2 contents were markedly increased by 160% and 207% upon exposure to 100 mM NaCl-salinity stress in leaves and roots, respectively, compared to the control seedlings (Figure 4A and 4B). Likewise, the MDA contents of the leaves and the roots were increased by 94% and 130% (Figure 4C and 4D). Conversely, the use of AsA partially alleviated the salt-induced oxidative damage and reduced the H_2O_2 and MDA contents, independently of its method of application. H_2O_2 and MDA contents were decreased by 21% and 19%, as well as by 20% and 22% in leaves and roots, respectively, following both the AsA/P and AsA/F treatments. However, significantly higher levels of decrease were obtained upon the AsA/R treatment, especially in roots, for which the H_2O_2 and MDA contents were almost restored to the levels of those of control seedlings (Figure4).



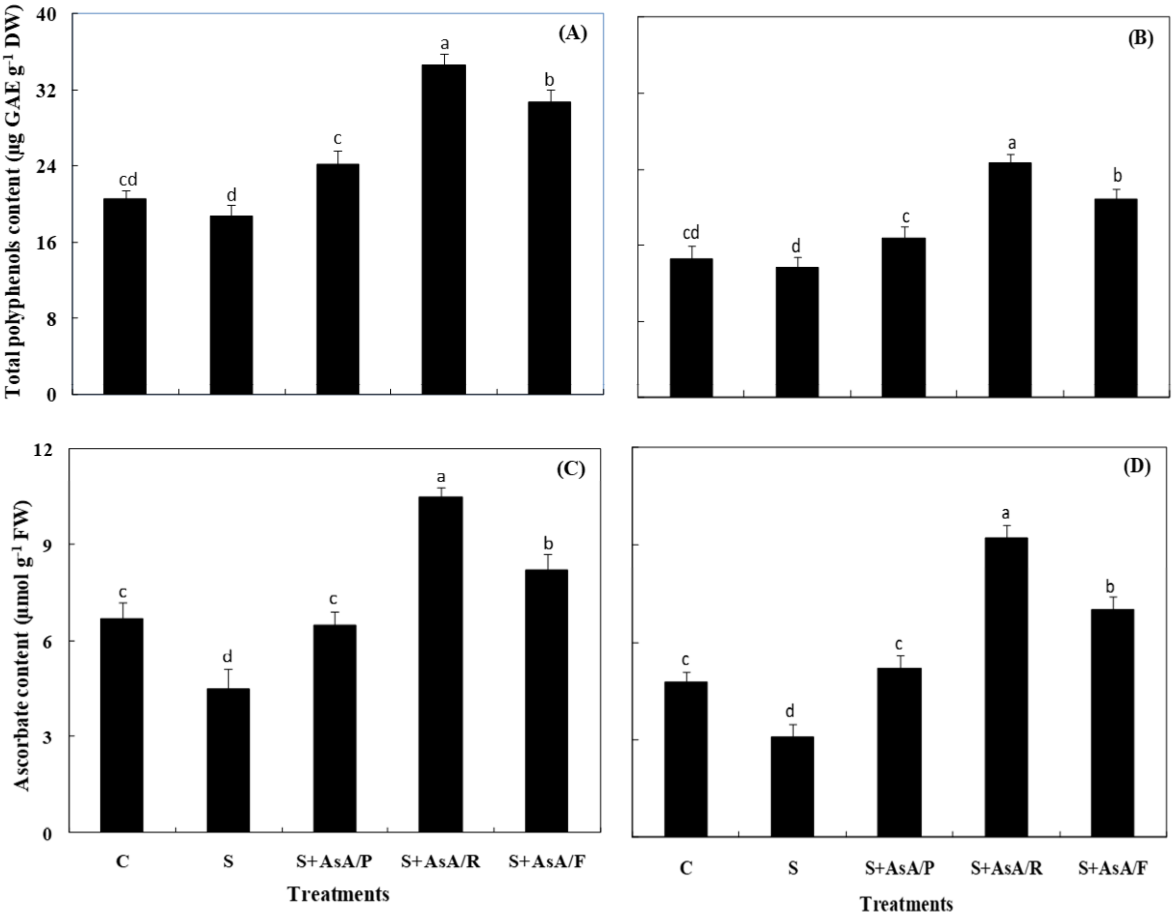


*Treatments were: (1) control (C, without NaCl and AsA treatments), (2) salinity (S, treatment with 100 mM NaCl), (3) salinity + AsA applied through seed priming (S + AsA/P), (4) salinity + AsA applied through the rooting medium (S + AsA/R), and (5) salinity + AsA applied through foliar spraying (S + AsA/F). Values are the means of six replicates ± S.D. Significantly different values are followed by different letters according to the one-way ANOVA test at $p < 0.05$.

Figure 4. *Hydrogen peroxide (H_2O_2) and malondialdehyde (MDA) contents in leaves (A, C) and roots (B, D) of Solanum lycopersicum cv. Rio Grande seedlings grown for two weeks under 100 mM NaCl-salinity and 1 mM ascorbic acid (AsA).*

Effects of AsA treatments on the non-enzymatic antioxidants of salt-stressed tomato seedlings

The results of this study showed that total polyphenol contents were not affected by salt stress (Figure 5A and 5B); however, ascorbate contents were decreased in salt-stressed tomato seedlings both in leaves and roots (Figure 5C and 5D), contrasted with the control seedlings. As expected, exogenous application of AsA increased the endogenous levels of AsA in leaves and roots of salt-stressed tomato seedlings. Nevertheless, such an accumulation was more pronounced with the AsA/R, compared to the AsA/F and AsA/P methods. Indeed, with the AsA/R method, leaves' and roots' AsA contents were enhanced by 133% and 197%, respectively, as compared to salt-treated seedlings. However, lower increases of 82% and 126%, as well as 44% and 67% were obtained in leaves and roots, respectively, following the AsA/F and AsA/P methods (Figure 5C and 5D). Likewise, total polyphenol contents were noticeably increased in salt-treated tomato seedlings, regardless of the method of AsA application. The highest levels of increase were observed following the AsA/R (by 85% and 80% in leaves and roots, respectively) followed by the AsA/F (by 64% and 52% in leaves and roots, respectively), whereas the lowest levels of increase were obtained with the AsA/P method (by 29% and 23% in leaves and roots, respectively) (Figure 5A and 5B).

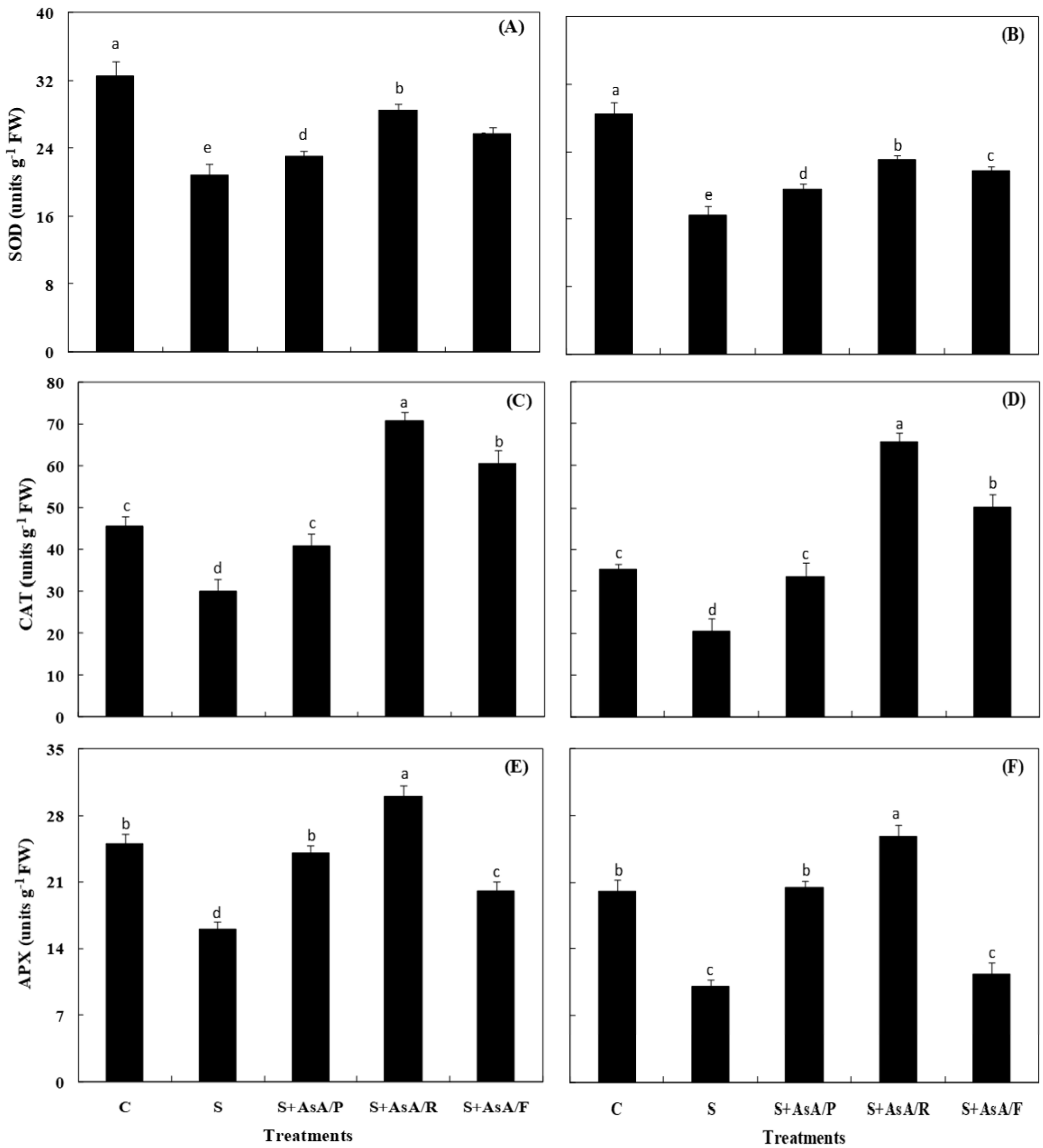


*Treatments were: (1) control (C, without NaCl and AsA treatments), (2) salinity (S, treatment with100 mM NaCl), (3) salinity + AsA applied through seed priming (S + AsA/P), (4) salinity + AsA applied through the rooting medium (S + AsA/R), and (5) salinity + AsA applied through foliar spraying (S + AsA/F). Significantly different values are followed by different letters according to the one-way ANOVA test at $p < 0.05$.

Figure 5. *Total polyphenols (A, B) and ascorbate (C, D) contents in leaves (A, C) and roots (B, D) of Solanum lycopersicum cv. Rio Grande seedlings grown for two weeks under 100 mM NaCl-salinity and 1 mM ascorbic acid (AsA).*

Effects of AsA treatments on the enzymatic antioxidants of salt-stressed tomato seedlings

As shown in Figure. 6, salt stress decreased the activities of SOD, CAT and APX by 36% and 43%, 33% and 42%, as well as 36% and 50% in leaves and roots, respectively. Except for the roots' APX activities following the AsA/F treatment, for which no effect was observed, all other AsA-application methods noticeably increased all analyzed antioxidant enzyme activities. As compared to those of AsA-untreated seedlings, SOD activities were enhanced by 37% and 41%, 24% and 32%, as well as 11% and 19% in leaves and roots, respectively, upon AsA/R, AsA/F and AsA/P treatments (Figure 6A and 6B). Similarly, CAT activities were enhanced by 136% and 222%, 100% and 146%, as well as 36% and 64% in leaves and roots, respectively (Figure 6C and 6D). In the same way, leaves and roots' APX activities were increased by 88% and 158%, as well as 50% and 104% following the AsA/R and AsA/P methods; whereas only a slight increase of 25% was observed in leaves' APX activity upon AsA/F treatment (Figure 6E and 6F).



*Treatments were: (**1**) control (C, without NaCl and AsA treatments), (**2**) salinity (S, treatment with 100 mM NaCl), (**3**) salinity + AsA applied through seed priming (S + AsA/P), (**4**) salinity + AsA applied through the rooting medium (S + AsA/R), and (**5**) salinity + AsA applied through foliar spraying (S + AsA/F). Values are the means of six replicates ± S.D. Significantly different values are followed by different letters according to the one-way ANOVA test at $p < 0.05$.

Figure 6. *Superoxide dismutase (SOD), catalase (CAT), and ascorbate peroxidase (APX) activities in leaves (A, C, E) and roots (B, D, F) of Solanum lycopersicum cv. Rio Grande seedlings grown for two weeks under 100 mM NaCl-salinity and 1 mM ascorbic acid (AsA).*

DISCUSSION

Plant growth is directly influenced by the surrounding environmental conditions. Under salt stress, plants experience growth and development inhibition as the most critical response to the adverse stress conditions (Zhao *et al.*, 2021). In this regard, findings of the current study demonstrated that all measured growth traits, including DW production, RWC, and LA, were noticeably reduced by salt stress (Table 2). The decrease in growth-related parameters in response to salt stress was previously reported in Super Marmande (Manaa *et al.*, 2014) and Agata (Horchani *et al.,*2025) tomato cultivars and could be explained by the disturbance of the reactions of carbon assimilation and the breakdown of essential ions uptake and assimilation, as well as the salt-induced physiological drought (Zhou *et al.*, 2024).

The ameliorating effect of exogenously applied-AsA was previously reported in many plant species, such as *Vicia faba* (Younis *et al.*, 2010), *Trifolium alexandrinum* (Aly *et al.*, 2012), *Hordeum vulgare* (Hassan *et al.*, 2021), and *Triticum durum* (Horchani *et al.*, 2024). The AsA-mediated protection could be explained by its role as a signal molecule in the activation of the osmoregulation and the ROS-scavenging processes, as well as the regulation of the ion homeostasis within plant organs (Hassan *et al.*, 2021). The results of the present study indicated that the application of AsA through the rooting medium is more efficient in the alleviation of the adverse effects of 100 mM NaCl-salinity on the growth of tomato seedlings, compared to the foliar spraying and seed priming methods (Table 2). In literature, the salinity tolerance of a plant has been positively correlated to the endogenous concentrations of AsA within its organs (Ajila-Celi *et al.*, 2025). Based on this observation and considering the higher AsA contents in roots and leaves of salt-stressed tomato seedlings following the AsA/R, in comparison to the AsA/F and AsA/P methods (Figure. 5C, 5D), it can be hypothesized that the effectiveness of the AsA/R method could be related not only to a higher entry of AsA in plant roots but also to a greater transport to the aerial part, compared to the AsA/F and AsA/P methods.

It is well known that the chlorophyll content of a plant is generally positively correlated to its photosynthetic activity and can be hence used as a reliable criterion to distinguish between salt-tolerant and sensitive plant species (Sairam *et al.*, 2002). Herein, the results indicated that salt stress significantly decreased total leaf chlorophyll content, indicating the sensitivity of the Rio Grande tomato cultivar to salt stress (Figure 1). Such a reduction is likely due to the disturbance of chlorophyll synthesis and/or acceleration of its degradation (Sairam *et al.*, 2002). Regardless of its method of application, AsA significantly increased the total chlorophyll content of salt-stressed tomato seedlings (Figure1). The results in this work are consistent with those obtained by Chen *et al.* (2024), who showed that AsA application increased the chlorophyll content of salt-treated tomato plants cv. Ligeer 87-5. Such an effect could be achieved through the enhancement of the chlorophyll biosynthesis pathway (Chen *et al.*, 2024), inhibition of the chlorophyllase enzyme (El-Hawary *et al.*, 2023), and/or the protective role of AsA against oxidative stress (Sairam *et al.*, 2002). Interestingly, the results of the current study showed that the level of increase in chlorophyll content depended on the manner of application of AsA, with the AsA/R and AsA/F treatments being more efficient in the mitigation of the effects of salt stress on photosynthesis pigments, in comparison to the AsA/P method.

As already reported in many other plant species (Cheesman, 1993; Fu & Huang, 2001; Horchani *et al.*, 2023; 2024), the results showed that salt stress significantly reduced total carbohydrate content (Figure2). Such a decrease could be attributed to the reduction of the photosynthetic activities which in turn could be due to the decrease of the chlorophyll content, and the inhibition of the Rubsico enzyme, as well as to the decrease in the stomatal conductance (Zhao *et al.*, 2021). Several studies showed that soluble sugars play a crucial role in the stabilization of the cell membrane and protoplast (Guo *et al.*, 2022) and also constitute an important source of carbon and energy that are essential for other organic synthesis processes and hence in plant growth (Hofius & Bornke, 2007; Miao *et al.*, 2020). Herein, the results indicated that AsA significantly increased total carbohydrate contents in leaves and roots, independently of its method of application (Figure 2). The higher increase in the total carbohydrate contents obtained with the AsA/R method (Figure2) could be a possible explanation of the significant amelioration of the growth of salt-stressed tomato seedlings following this manner of AsA application (Table 1).

Proline is an osmoprotectant molecule routinely used as an indicator of the plant tolerance to salt stress (Agami, 2014). In this study, the significant decrease in the content of such an osmoticum (Figure 3) confirms the sensitivity of this tomato cultivar to salt stress (Misra & Saxena, 2009) and might be due to decreased activities of enzymes involved in proline biosynthesis pathway (mainly delta-1-pyrroline-5-carboxylate synthaseand pyrroline-5-carboxylate reductase), and/or to increased activities of enzymes involved in proline catabolism (such as proline dehydrogenase and pyrroline 5-carboxylase dehydrogenase) (Hosseinifard *et al.*, 2022). Independent of its method of application, AsA noticeably increased the proline content of salt-stressed tomato seedlings (Figure 3), with a concomitant increase in the RWC (Table 1). Taken together, these results confirm the role of AsA in the mitigation of the salt-induced osmotic stress. The AsA-induced increase in proline content has been previously reported in many salt-stressed plants such as cucumber (Seleiman *et al.*, 2020), tomato (Chen *et al.*, 2021), and barley (Horchani *et al.*, 2024). Interestingly, the results of the current study indicated that the AsA/R method was more efficient in the enhancement of osmoprotectant synthesis, as compared to the AsA/F and AsA/P methods (Figure 3).

It is known that salt stress generates a secondary oxidative stress through excessive ROS production (Xu *et al.*, 2022). These oxidizing compounds provoke serious damage to lipids, proteins, and deoxyribonucleic acid, thereby increasing damage to membrane integrity and hence cellular dysfunction (Rahman *et al.*, 2022). Similarly, it has been shown for many other crop plants, such as *Zea mays* (Abd-Elgawad *et al.,* 2016) and *Triticum aestivum* (Horchani *et al.*, 2024). The findings of the present study showed that salt treatment increased the generation of free radical H_2O_2, causing more significant damage to membrane lipids as indicated by the significant increase in leaves' and roots' MDA contents (Figure 4). Several studies showed that AsA applied to salt-stressed plants acts as ROS-scavenger and contribute hence to the maintenance of cell membrane integrity and functions (Rahman *et al.*, 2022). In line with this, the results of this study indicated that AsA mitigated the salt-induced oxidative stress as indicated by the significant decreases in leaves and roots' H_2O_2 and MDA contents (Figure 4). However, the AsA/R method was found to be more efficient compared to the AsA/F and AsA/P methods (Figure 4).

To alleviate the adverse effects of salt stress on the structure and functioning of the essential macromolecules and hence on growth, plants activate the antioxidant system for mediating the fast scavenging of toxic ROS (Alnusairi *et al.*, 2021; Horchani *et al.*, 2023; 2024). In this study, this scenario did not seem to have occurred, since total polyphenols were not affected (Figure 5); however, ascorbate contents as well as SOD, CAT, and APX activities (Figure 6) were decreased in salt-stressed tomato seedlings, contrasted with the controls. Such a result could be explained by the increase in the magnitude of the oxidative stress and cell injury in tomato seedlings due to the overproduction of ROS (Nasrallah *et al.*, 2022). Polyphenols and AsA are known as efficient non-enzymatic antioxidants involved in ROS scavenging; therefore, their accumulation in a plant could be an indicator of its tolerance to salt stress (Nazar *et al.*, 2015). In this study, exogenous application of AsA increased the endogenous levels of AsA and total polyphenols in leaves and roots of salt-stressed tomato seedlings (Figure 5), indicating the role of this PGR in the amelioration of the tolerance of tomato seedlings to salt stress, particularly when applied through the rooting medium. Furthermore, to scavenge ROS, namely H_2O_2, plants activate a range of antioxidant enzymes (Rahman *et al.*, 2022). The results indicated that salt-stressed tomato seedlings exhibited significant increases in CAT, SOD and APX activities upon different modes of treatment with AsA (Figure 6). These results are in accordance with those of Horchani *et al.* (2024) in barley and Kanwal *et al.* (2024) in pea who showed that AsA applied through the rooting medium and foliar spraying, respectively, boosted the SOD and CAT activities. Interestingly, data of the present study showed that such an effect was more pronounced with the AsA/R compared to the AsA/F and AsA/P methods (Figure 5). The significant increases in the SOD, CAT and APX activities, as well as total polyphenols and AsA contents upon the AsA/R compared to the AsA/F and AsA/P methods are explanations to the substantial decreases in the H_2O_2 and MDA contents, and hence to the effectiveness of this AsA-application method in the mitigation of the salt-induced devastating effects.

CONCLUSIONS

Overall, the findings of the current study showed that salt stress reduced the growth of tomato seedlings cv. Rio Grande. This reduction is associated with reduced total chlorophyll and total carbohydrate contents, increased oxidative damage, as well as reduced proline accumulation and disrupted antioxidant system. Regardless of the method of application, AsA had positive effects on almost all the parameters that were reduced by salt stress. The AsA-ensured protection of tomato seedlings against salt stress was achieved through the accumulation of chlorophyll, carbohydrates, and proline, and the decrease of the oxidative damage, as well as the upregulation of the non-enzymatic and enzymatic antioxidant system. Additionally, the results of the current study showed that the AsA-application method is a key factor influencing its effectiveness under salt stress conditions. The root application method was found to be more efficient in alleviating the inhibitory effect of salt stress on the growth and on all the studied biochemical characteristics of tomato seedlings, compared to the foliar spraying and seed priming methods. This study highlights the promising application of AsA through the rooting medium as an effective method for improving tomato growth and physio-biochemical attributes in salt-affected soils, offering a sustainable solution for saline agriculture.

AUTHOR CONTRIBUTIONS

All authors contributed to the study conception and design. Material preparation, data collection, and analysis were performed by FH, AB, AB, and ZA. The first draft of the manuscript was written by FH, and all authors commented on previous versions of the manuscript. All authors read and approved the final version of the manuscript.

FUNDING

The authors did not receive support from any organization for the submitted work.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

REFERENCES

Aazami, M. A.; Rasouli, F.; Ebrahimzadeh, A. (2021). Oxidative damage, antioxidant mechanism and gene expression in tomato responding to salinity stress under in vitro conditions and application of iron and zinc oxide nanoparticles on callus induction and plant regeneration. *BMC Plant Biology*. 21(1): 597. https://doi.org/10.1186/s12870-021-03379-7

Abd-Elgawad, H.; Zinta, G.; Hegab, M. M.; Pandey, R.; Asard, H.; Abuelsoud, W. (2016). High salinity induces different oxidative stress and antioxidant responses in maize seedlings organs. *Frontiers in Plant Science*. 7: 276. https://doi.org/10.3389/fpls.2016.00276

Agami, R. A. (2014). Applications of ascorbic acid or proline increase resistance to salt stress in barley seedlings. *BiologiaPlantarum*. 58: 341-347. https://doi.org/10.1007/s10535-014-0392-y

Ajila-Celi, G. E.; Lata-Tenesaca, L. F.; Calzada, K. P.; Alves, R. C.; da Cruz, M. C. P.; Junior, J. S. P.; Carrega, W. C.; Gratão, P. L. (2025). Exogenous ascorbic acid mitigates salt-induced damage in soybean by modulating photosynthesis, antioxidant defense, and ionic homeostasis. *Acta Physiologiae Plantarum*. 47:26 https://doi.org/10.1007/s11738-025-03770-z

Alnusairi, G. S.; Mazrou, Y. S.; Qari, S. H.; Elkelish, A. A.; Soliman, M. H.; Eweis, M.; Abdelaal, K.; El-Samad, G. A.; Ibrahim, M. F.; El-Nahhas, N. (2021). Exogenous nitric oxide reinforces photosynthetic efficiency, osmolyte, mineral uptake, antioxidant, expression of stress-responsive genes and ameliorates the effects of salinity stress in wheat. *Plants*. 10(8): 1693. https://doi.org/10.3390/plants10081693

Aly, A. A.; Khafaga, A. F.; Omar G. N. (2012). Improvement the adverse effect of salt stress in Egyptian clover (*Trifoliumalexandrinum* L.) by ascorbic acid application through some biochemical and RT-PCR markers. *Journal of Applied Phytotechnology in Environmental Sanitation*. 1(2): 91-102.

Arif, Y.; Singh, P.; Siddiqui, H.; Bajguz, A.; Hayat, S. (2020). Salinity induced physiological and biochemical changes in plants: An omic approach towards salt stress tolerance. *Plant Physiology and Biochemistry*. 156: 64-77. https://doi.org/10.1016/j.plaphy.2020.08.042

Arnon, D. I. (1949). Copper enzymes in isolated chloroplasts. Polyphenol oxidase in *Beta* *vulgaris*. *Plant Physiology*. 24(1): 1-15. https://doi.org/10.1104/pp.24.1.1

Bates, L. S.; Waldren, R. P.; Teare, I. (1973). Rapid determination of free proline for water stress studies. *Plant and Soil*. 39: 205-207. https://doi.org/10.1007/BF00018060

Billah, M.; Rohman, M. M.; Hossain, N.; Uddin, M. S. (2017). Exogenous ascorbic acid improved tolerance in maize (*Zea mays* L.) by increasing antioxidant activity under salinity stress. *African Journal of Agricultural Research*.12(17): 1437-1446. https://doi.org/10.5897/AJAR2017.12295

Cheesman, J. M. (1993). Plant growth modelling without integrating mechanisms. *Plant Cell & Environment*.16(2): 137-147. https://doi.org/10.1111/j.1365-3040.1993.tb00855.x

Chen, X.; Han, H.; Cong, Y.; Li, X.; Zhang, W.; Wan, W.; Cui, J.; Xu, W.; Diao, M.; Liu H. (2023). The protective effect of exogenous ascorbic acid on photosystem inhibition of tomato seedlings induced by salt stress. *Plants*. 12(6): 1379. https://doi.org/10.3390/plants12061379

Chen, X.; Jiang, Y.; Cong, Y.; Liu, X.; Yang, Q.; Xing, J.; Liu, H. (2024). Ascorbic acid mitigates salt stress in tomato seedlings by enhancing chlorophyll synthesis pathways. *Agronomy*. 14(8): 1810. https://doi.org/10.3390/agronomy14081810

Chen, X.; Zhou, Y.; Cong, Y.; Zhu, P.; Xing, J.; Cui, J.; Xu, W.; Shi, Q.; Diao, M.; Liu, H. (2021). Ascorbic acid-induced photosynthetic adaptability of processing tomatoes to salt stress probed by fast OJIP fluorescence rise. *Frontiers in Plant Sciences*. 12: 594400. https://doi.org/10.3389/fpls.2021.594400

El-Hawary, M. M.; Hashem, O. S. M.; Hasanuzzaman. M. (2023). Seed priming and foliar application with ascorbic acid and salicylic acid mitigate salt stress in wheat. *Agronomy*. 13(2): 493. https://doi.org/10.3390/agronomy13020493

El-Sabagh, A.; Islam, M. S.; Skalick, M.; Raza, M. A.; Singh, K.; Anwar Hossain, M.; Hossain, A.; Mahboob, W.; Iqbal, M. A.; Ratnasekera, D.; Singhal, R. K.; Ahmed, S.; Kumari, A.; Wasaya, A.; Sytar, O.;Brestic, M.; Çig, F.; Erman, M.; Habib Ur Rahman, M., Ullah, N.; Arshad, A. (2021). Salinity stress in wheat (*Triticum aestivum* L.) in the changing climate: adaptation and management strategies. *Frontiers in Agronomy*. 3: 661932. https://doi.org/10.3389/fagro.2021.661932

Fu, J.; Huang, B. (2001). Involvement of antioxidant and lipid peroxidation in the adaptation of two cool-season grasses to localized drought stress. *Environmental and Experimental Botany*. 45(2): 105-114. https://doi.org/10.1016/S0098-8472(00)00084-8

Giannopolitis, C. N.; Ries, S. K. (1977). Superoxide dismutase. I. Occurrence in higher plants. *Plant Physiology*. 59(2): 309-314. https://doi.org/10.1104/pp.59.2.309

Guo, S.; Ma, X.; Cai, W.; Wang, Y.; Gao, X.; Fu, B.; Li, S. (2022). Exogenous proline improves salt tolerance of alfalfa through modulation of antioxidant capacity, ion homeostasis, and proline metabolism. *Plants*. 11(21): 2994. https://doi.org/10.3390/plants11212994

Hasanuzzaman, M.; Raihan, M. R. H.; Alharby, H. F.; Al-Zahrani, H. S.; Alsamadany, H.; Alghamdi, K. M.; Ahmed, N.; Nahar, K. (2023). Foliar application of ascorbic acid and tocopherol in conferring salt tolerance in rapeseed by enhancing K^+/Na^+ homeostasis, osmoregulation, antioxidant defense, and glyoxalase system. *Agronomy*.13(2): 361. https://doi.org/10.3390/agronomy13020361

Hassan, A.; Fasiha, A. S.; Hamzah, S. M.; Yasmin, H.; Imran, M.; Riaz, M.; Ali, Q.; Ahmad, J. F.; Mobeen, A. S.; Ali, S.; Abdullah, A. A.; Nasser, A. M. (2021). Foliar application of ascorbic acid enhances salinity stress tolerance in barley (*Hordeum vulgare* L.) through modulation of morpho-physio-biochemical attributes, ions uptake, osmo-protectants and stress response genes expression. *Saudi Journal of Biological Sciences*. 28(8): 4276-4290. https://doi.org/10.1016/j.sjbs.2021.03.045

Hofius, D.; Bornke, F. (2007). Photosynthesis, carbohydrate metabolism and source-sink relations. In: Bradshaw, J.; Gebhardt, C.; Govers, F.; Mackerron, D. K. L.; Taylor, M.A.; Ross, H. A. *Potato Biology and Biotechnology*. pp.257-285. Elsevier Science. 823p. https://doi.org/10.1016/B978-044451018-1/50055-5

Horchani, F.; Aloui, A.; Brouquisse, R.; Aschi-Smiti, S. (2008). Physiological responses of tomato plants (*Solanum lycopersicum*) as affected by root hypoxia. *Journal of Agronomy and Crop Science*. 194(4): 297–303. https://doi.org/10.1111/j.1439-037X.2008.00313.x

Horchani, F.; Bouallegue, A.; Bouazzi, A.; Abbes, Z. (2025). Alleviating salt-induced effects in tomato via simultaneous application of salicylic acid and potassium. *Russian Journal of Plant Physiology*. 72: 8. https://doi.org/10.1134/S1021443724609261

Horchani, F.; Bouallegue, A.; Namsi, A.; Abbes, Z. (2023). Exogenous application of ascorbic acid mitigates the adverse effects of salt stress in two contrasting barley cultivars through modulation of physio-biochemical attributes, K^+/Na^+ homeostasis, osmoregulation and antioxidant defense system. *Russian Journal of Plant Physiology*. 70: 219. https://doi.org/10.1134/S1021443723602598

Horchani, F.; Bouallegue, A.; Namsi, A.; Abbes, Z. (2024). Simultaneous application of ascorbic acid and proline as a smart approach to mitigate the adverse effects of salt stress in wheat (*Triticum aestivum*). *Biology Bulletin*. 51(5): 1346-1363. https://doi.org/10.1134/S1062359024607171

Hosseinifard, M.; Stefaniak, S.; Ghorbani Javid, M.; Soltani, E.; Wojtyla, Ł.; Garnczarska, M. (2022). Contribution of exogenous proline to abiotic stresses tolerance in plants: A Review. *International Journal of Molecular Sciences*. 23(9): 5186. https://doi.org/10.3390/ijms23095186

Jha, U.; Bohra, A.; Jha, R.; Parida, S. K. (2019). Salinity stress response and 'omics' approaches for improving salinity stress tolerance in major grain legumes. *Plant Cell Reports*. 38(3): 255-277.

Junglee, E. S.; Urban, L.; Sallanon, H; Lopez, I. (2014). Optimized assay for hydrogen peroxide determination in plant tissue using potassium iodide. *American Journal of Analytical Chemistry*. 5(11): 730-736. https://doi.org/10.4236/ajac.2014.511081

Kanwal, R.; Maqsood, M.F.; Shahbaz, M.; Naz, N.; Zulfiqar, U.; Muhammad, F. A.; Jamil, M.; Khalid, F.; Qasim, A.; Muhammad, A. S.; Talha C.; Hayssam M. A.; Waleed A. A. A. (2024). Exogenous ascorbic acid as a potent regulator of antioxidants, osmo-protectants, and lipid peroxidation in pea under salt stress. *BMC Plant Biology*. 24: 247.https://doi.org/10.1186/s12870-024-04947-3

Liu, H.; Meng, F.; Miao, H.; Chen, S.; Yin, T.; Hu, S.; Shao, Z.; Liu, Y.; Gao, L.; Zhu, C.; Zhang, B.; Wang, Q. (2018). Effects of postharvest methyl jasmonate treatment on main health-promoting components and volatile organic compounds in cherry tomato fruits. *Food Chemistry*.263: 194-200. https://doi.org/10.1016/j.foodchem.2018.04.124

Manaa, A.; Gharbi, E.; Mimouni, H.; Wasti, S.; Aschi-Smiti, S.; Lutts, S.; Ben Ahmed, H. (2014). Simultaneous application of salicylic acid and calcium improves salt tolerance in two contrasting tomato (*Solanum lycopersicum*) cultivars. *SouthAfrican Journal of Botany*. 95: 32-39. https://doi.org/10.1016/j.sajb.2014.07.015

McCready, R. M.; Guggolz, J.; Silviera, V.; Owes, H. S. (1950). Determination of starch and amylase in vegetables, application to peas. *Analytical Chemistry*. 22(9): 1156-1158. https://doi.org/10.1021/ac60045a016

Miao, Y.; Luo, X.; Gao, X.; Wang, W.; Li, B.; Ho, L. (2020). Exogenous salicylic acid alleviates salt stress by improving leaf photosynthesis and root system architecture in cucumber seedlings. *Scientia Horticulturae*. 272: e109577. https://doi.org/10.1016/j.scienta.2020.109577

Misra, N.; Saxena, P. (2009). Effect of salicylic acid on proline metabolism in lentil grown under salinity stress. *Plant Science*. 177(3): 181-189. https://doi.org/10.1016/j.plantsci.2009.05.007

Nasrallah, A. K.; Kheder, A. A.; Kord, M. A.; Fouad, A. S.; El-Mogy, M. M.; Atia, M. A. (2022). Mitigation of salinity stress effects on broad bean productivity using calcium phosphate nanoparticles application. *Horticulturae*. 8(1): 75. https://doi.org/10.3390/horticulturae8010075

Nazar, R.; Umar, S.; Khan N. A. (2015). Exogenous salicylic acid improves photosynthesis and growth through increase in ascorbate-glutathione metabolism and S assimilation in mustard under salt stress. *Plant Signaling and Behavior*. 10(3): e1003751. https://doi.org/10.1080/15592324.2014.1003751

Njus, D.; Kelley, P. M.; Tu, Y. J.; Schlegel, H. B. (2020). Ascorbic acid: The chemistry underlying its antioxidant properties. *Free Radical Biology and Medicine*. 159: 37-43. https://doi.org/10.1016/j.freeradbiomed.2020.07.013

Rahman, A.; Alam, M. U.; Hossain, M. S.; Mahmud, J. A.; Nahar, K.; Fujita, M.; Hasanuzzaman, M. (2022). Exogenous gallic acid confers salt tolerance in rice seedlings: Modulation of ion homeostasis, osmoregulation, antioxidant defense, and methylglyoxal detoxification systems. *Agronomy*. 13(1): 16. https://doi.org/10.3390/agronomy13010016

Sairam, R. K.; Veerabhadra-Rao, K.; Srivastava, G. C. (2002). Differential response of wheat genotypes to long term salinity stress in relation to oxidative stress, antioxidant activity and osmolyte concentration. *Plant Science*. 163(5): 1037-1046. https://doi.org/10.1016/S0168-9452(02)00278-9

Sarker, U.; Oba S. (2020). The response of salinity stress-induced *A. tricolor* to growth, anatomy, physiology, non-enzymatic and enzymatic antioxidants. *Frontiers in Plant Science*. 11: 559876. https://doi.org/10.3389/fpls.2020.559876

Seleiman, M. F.; Semida, W. M.; Rady, M. M.; Mohamed, G. F.; Hemida, K. A.; Alhammad, B. A.; Hassan, M. M.; Shami, A. (2020). Sequential application of antioxidants rectifies ion imbalance and strengthens antioxidant systems in salt-stressed cucumber. *Plants*. *9*(12): 1783. https://doi.org/10.3390/plants9121783

Silveira, L. F.; Olmos, F.; Rodaand, S. A.; Long, A. J. (2003). Notes on the seven-colouredTanagerTanagafastuosa in north-east Brazil. *Cotinga*. 20: 82-88.

Xu, L.; Chen, H.; Zhang, T.; Deng, Y.; Yan, J.; Wang, L. (2022). Salicylic acid improves the salt tolerance capacity of *Saponaria officinalis* by modulating its photosynthetic rate, osmoprotectants, antioxidant levels, and ion homeostasis. *Agronomy*. 12(6): 1443. https://doi.org/10.3390/agronomy12061443

Younis, M. E.; Hasaneen, M. N.; Kazamel, A. M. (2010). Exogenously-applied ascorbic acid ameliorates detrimental effects of NaCl and mannitol stress in *Viciafaba* seedlings. *Protoplasma*. 239: 39-48. https://doi.org/10.1007/s00709-009-0080-5

Zarai, B.; Walter, C.; Michot, D.; Montoroi, J. P.; Hachicha, M. (2022). Integrating multiple electromagnetic data to map spatiotemporal variability of soil salinity in Kairouan region, Central Tunisia. *Journal of Arid Lands*. 14: 186-202. https://doi.org/10.1007/s40333-022-0052-6

Zhao, S.; Zhang, Q.; Liu, M.; Zhou, H.; Ma, C.; Wang, P. (2021). Regulation of plant responses to salt stress. *International Journal of Molecular Sciences*. 22(9): 4609. https://doi.org/10.3390/ijms22094609