

Foliar Application of Ascorbic Acid, Tocopherol and Arginine Improves Photosynthetic Performance and Water Relations of Maize under Naturally Saline Field Conditions

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ABSTRACT

Salinity is a major abiotic stress that restricts maize growth by reducing photosynthetic efficiency, disturbing plant water relations, and triggering oxidative damage. Although natural biostimulants have been widely studied for improving plant stress tolerance, evidence regarding the combined effects of ascorbic acid, tocopherol, and arginine under saline field conditions remains limited. This study evaluated the efficacy of individual and combined foliar applications of these biostimulants in enhancing the physiological and biochemical responses associated with salinity tolerance in maize cultivated in naturally saline soils. Field experiments were conducted during the 2023 and 2024 growing seasons, and each biostimulant was applied at 1 g L⁻¹ either separately or in combination. Salinity stress markedly decreased photosynthetic pigment concentrations, gas-exchange attributes, and plant water status. However, the combined treatment consistently produced superior responses compared with the individual applications, significantly improving photosynthetic performance, water relations, and osmotic adjustment. These improvements were reflected in higher chlorophyll content, net photosynthetic rate, proline accumulation, and relative water content across both seasons. The combined treatment consistently produced greater improvements than the individual applications, particularly in chlorophyll content, net photosynthetic rate, relative water content, and proline accumulation, across both growing seasons. These findings indicate that the combined foliar application of ascorbic acid, tocopherol, and arginine is a promising approach for improving maize physiological performance under saline field conditions.

Keywords: chlorophyll, proline, relative water content, transpiration rate.

INTRODUCTION

Maize (*Zea mays* L.) is one of the world's most important cereal crops, providing food, feed, and industrial raw materials. However, its productivity is severely constrained by soil salinity, particularly in arid and semi-arid regions, where excessive salt accumulation disrupts ion homeostasis, limits water uptake, impairs photosynthesis, and induces oxidative stress, ultimately reducing plant growth and grain yield

(Corwin and Scudiero, 2019; Hasanuzzaman et al., 2021; Seleiman et al., 2022).

Foliar application of natural biostimulants has emerged as an environmentally sustainable approach for improving plant tolerance to salinity stress. Among these, ascorbic acid (AsA), tocopherol (Toc), and arginine (Arg) enhance antioxidant defense, stabilize chloroplast and cellular membranes, regulate ion homeostasis, promote osmotic adjustment, and preserve photosynthetic efficiency through complementary physiological mechanisms

(Sabagh et al., 2021; Hasanuzzaman et al., 2023; Sadaf et al., 2025). Nevertheless, previous studies have primarily evaluated these compounds individually, while information regarding their combined application and potential synergistic effects under naturally saline field conditions remains limited.

Therefore, this study investigated the effects of individual and combined foliar applications of AsA, Toc, and Arg on the physiological and biochemical responses of maize grown under naturally saline soil conditions. We hypothesized that the combined application of these complementary biostimulants would produce synergistic improvements in photosynthetic performance, plant water relations, osmotic adjustment, and overall salinity tolerance compared with their individual application.

MATERIAL AND METHODS

The experiment was conducted in South Port Said, Egypt (31°08'20" N, 32°17'29" E),

during two consecutive summer growing seasons (2023 and 2024). Throughout the experimental period, the average air temperature ranged from 26 to 27°C, while relative humidity ranged from 65% to 70%. The study area is characterized by high solar radiation, with an average of 11-12 h of sunshine per day, corresponding to approximately 23-26 MJ m⁻² day⁻¹.

The experimental material consisted of the yellow maize hybrid SC 168, which was obtained from the Cereal Crops Department, Field Crops Research Institute, Agricultural Research Center (ARC), Giza, Egypt. Alfalfa (*Trifolium alexandrinum* L.) was grown as the preceding crop in both seasons. Prior to the establishment of the experiment, surface soil samples (0-20 cm depth) were collected for chemical characterization. Soil chemical properties were determined according to the methods described by Dane and Topp (2020) and Sparks et al. (2020), and the results are presented in Table 1.

Table 1. Mean values of selected chemical soil properties at the experimental site prior to planting during the two growing seasons

Seasons	EC	PH	Texture	Cations (ml equ/L)				Anions (ml equ/L)			
				Ca ⁺²	Mg ⁺	Na ⁺	K ⁺	CO ₃ ⁻	HCO ₃ ⁻	Cl ⁻	SO ₄ ⁻
2023	7.6	8.68	clayey	9.88	11.64	63.1	2.29	-	1.1	64.9	21.09
2024	7.2	8.94	clayey	10.12	12.03	64.1	2.41	-	1.03	65.21	22.42

The soil was plowed two weeks before sowing and subsequently leveled using laser-guided land-leveling technology. The experiment was arranged in a randomized complete block design (RCBD) with five treatments and five replications in each growing season. Each experimental plot consisted of five ridges, each 3 m in length and spaced 70 cm apart, resulting in a total plot area of 10.5 m².

Seeds were sown on May 25, 2023, and May 30, 2024, in hills spaced 25 cm apart under naturally saline soil conditions, as presented in Table 1. Seedling emergence was monitored regularly, and thinning was performed 21 days after sowing, leaving one plant per hill. Plants were harvested at the beginning of September in both growing seasons.

Phosphorus fertilizer was applied at a rate of 36 kg P₂O₅ ha⁻¹ during soil preparation in the form of calcium superphosphate (15.5% P₂O₅). Nitrogen fertilizer was supplied at a rate of 288 kg N ha⁻¹ as urea (46% N) and was applied in three equal split doses before irrigation. Irrigation water was obtained from the Nile River. All other agronomic practices were performed according to the standard recommendations for maize cultivation in the region.

Treatments

The experiment was conducted under naturally saline field conditions, with soil electrical conductivity (EC) values of 7.6 and 7.2 dS m⁻¹ during the 2023 and 2024 growing seasons, respectively. These values represented

a naturally occurring salinity level that remained relatively stable throughout the study. No artificial salinity treatments or salinity gradients were imposed.

Foliar treatments consisted of ascorbic acid (AsA; 99% purity, Sigma-Aldrich, USA), tocopherol (Toc; $\geq 97\%$ purity, Sigma-Aldrich, USA), and arginine (Arg; $\geq 98\%$ purity, Sigma-Aldrich, USA). Each compound was applied at a concentration of 1 g L^{-1} either individually or in combination under naturally saline field conditions. Foliar applications were performed twice, at 25 and 40 days after sowing (DAS), using a hand-held sprayer until runoff.

Five treatments were evaluated: (1) untreated control (distilled water spray), (2) AsA at 1 g L^{-1} , (3) Toc at 1 g L^{-1} , (4) Arg at 1 g L^{-1} , and (5) a combined application of AsA, Toc, and Arg, each at 1 g L^{-1} . Control plants were sprayed with an equivalent volume of distilled water at the same application times.

Chlorophyll and carotenoids content

Photosynthetic pigment contents, including chlorophylls and carotenoids [mg g^{-1} fresh weight (FW)], were determined using fresh leaf samples collected from the third fully expanded leaf from the apex at 65 days after sowing (DAS). Leaf tissue (0.5 g) was homogenized in 50 mL of acetone using a tissue homogenizer. The homogenate was centrifuged at 10,000 rpm for 15 min at 4°C . The resulting extract was then filtered through a $0.45 \text{ }\mu\text{m}$ nylon syringe filter, and the clear filtrate was used for the spectrophotometric determination of chlorophyll a, chlorophyll b, and carotenoids using a Spectronic 20 Genesys spectrophotometer (Thermo Scientific, USA).

Absorbance values were recorded at 645 and 655 nm for chlorophyll (a and b) determination and at 480 and 495 nm for carotenoid determination. All measurements were performed in triplicate. Pigment concentrations were calculated according to the method described by Bulda et al. (2008) using the following equations:

$$\text{Chl. a} = 19.00 A_{655} - 7.61 A_{645}$$

$$\text{Chl. b} = 21.45 A_{645} - 5.92 A_{655}$$

$$C_{\beta\text{-car}} = 17.16 A_{495} - 3.96 A_{480}$$

where A is absorption of the solution at 655, 645, 480 and 495 nm, $C_{\beta\text{-car}}$ is the concentration of $\beta\text{-car}$ (mg/l).

Stomatal conductance, net photosynthesis and transpiration rate

Stomatal conductance, transpiration rate, and photosynthetic rate were determined using a steady-state porometer (LI-1600, LI-COR Inc., Lincoln, NE, USA) equipped with a lithium chloride sensor. Prior to measurements, the instrument was calibrated according to the procedure described by Krishnan et al. (2011). Measurements were taken from the fully expanded leaf of each replicate. Stomatal conductance and photosynthetic rate were expressed as $\mu\text{mol m}^{-2} \text{ s}^{-1}$, whereas transpiration rate was recorded as $\text{mmol m}^{-2} \text{ s}^{-1}$.

Proline and relative water content

Proline content was determined in samples collected from the second fully expanded leaf from the apex and expressed as $\mu\text{mol g}^{-1}$ fresh weight (FW) according to the method of Bates et al. (1973).

Plant dry weight was determined by drying plant samples in a forced-air oven at 70°C for 72 h until a constant weight was achieved and was expressed as g plant^{-1} .

Relative water content (RWC) was determined using leaf tissue samples based on their fresh weight (FW), turgid weight (TW), and dry weight (DW), according to the following equation:

$$\text{RWC (\%)} = \frac{\text{FW} - \text{DW}}{\text{TW} - \text{DW}} \times 100$$

where FW (Fresh Weight) refers to the weight of freshly collected leaf tissue. TW is the weight of the leaf tissue after soaking in distilled water until fully hydrated. DW represents the weight of the tissue after oven-drying. Plant samples were oven-dried at 70°C for 72 h until a constant weight was obtained before dry weight determination.

Statistical Analysis

Data were subjected to analysis of variance (ANOVA), and treatment effects were considered statistically significant at $p \leq 0.05$. All statistical analyses were performed using CoStat software (version

6.4; CoHort Software, Monterey, CA, USA). When significant differences among treatments were detected, mean comparisons were conducted using Duncan's Multiple Range Test (DMRT) at the 5% probability level according to Duncan (1955). Figures were prepared using GraphPad Prism (version 10.4.1).

RESULTS AND DISCUSSION

All measured parameters were subjected to analysis of variance (ANOVA), and treatment means were separated using Duncan's Multiple Range Test (DMRT) at the 5% significance level ($P \leq 0.05$). Unless otherwise indicated, differences among treatments discussed in the text were statistically significant at $P \leq 0.05$. In all figures, means followed by the same letter were not significantly different according to Duncan's Multiple Range Test.

Chlorophyll content

Analysis of variance revealed significant treatment effects ($P \leq 0.05$) on chlorophyll *a* and chlorophyll *b* contents in both growing seasons (Figures 1a and 1b). Overall, foliar application of the tested biostimulants enhanced chlorophyll accumulation compared with the untreated control, although the increase in chlorophyll *a* following AsA application during the first season was not statistically significant. The combined application of AsA, Toc and Arg consistently produced the highest chlorophyll *a* and *b* contents across both seasons, demonstrating a clear synergistic effect. Relative to the control, the combined treatment increased chlorophyll *a* by 65.4% and 34.7% and chlorophyll *b* by 55.3% and 52.9% in the 2023 and 2024 growing seasons, respectively. Among the individual treatments, AsA was the most effective, increasing chlorophyll *a* by 25.6% and 26.7% and chlorophyll *b* by 38.0% and 41.7% across the two seasons. In contrast, Arg exhibited the weakest response,

with corresponding increases of only 4.4-6.2% for chlorophyll *a* and 7.9-11.0% for chlorophyll *b*.

Carotenoids content

All foliar-applied biostimulants significantly increased carotenoid content compared with the untreated control (Figure 1c). The combined application of AsA, Toc, and Arg produced the greatest response, increasing carotenoid content by 126.4% and 96.9% during the 2023 and 2024 growing seasons, respectively. Among the individual treatments, AsA was the most effective, with increases of 88.0% and 72.0%, followed by Toc (63.0% and 49.0%) and Arg (47.1% and 29.7%) across the two seasons. Duncan's multiple range test confirmed the superiority of the combined treatment over all individual applications, while each individual biostimulant treatment also differed significantly from the untreated control, as indicated by the different letters assigned to treatment means (Figure 1c).

Net photosynthesis

All biostimulant treatments significantly enhanced the net photosynthetic rate compared with the untreated control (Figure 1d). The combined application of AsA, Toc and Arg produced the greatest improvement, increasing net photosynthesis by 37.4% and 40.8% during the 2023 and 2024 growing seasons, respectively. Among the individual treatments, AsA and Toc also significantly increased net photosynthetic rate, whereas Arg elicited the smallest response, with increases of 11.5% and 11.1% across the two seasons. Duncan's multiple range test confirmed that the combined treatment was significantly superior to all individual applications. These results indicate a synergistic effect of the combined biostimulants in enhancing photosynthetic performance under naturally saline field conditions.

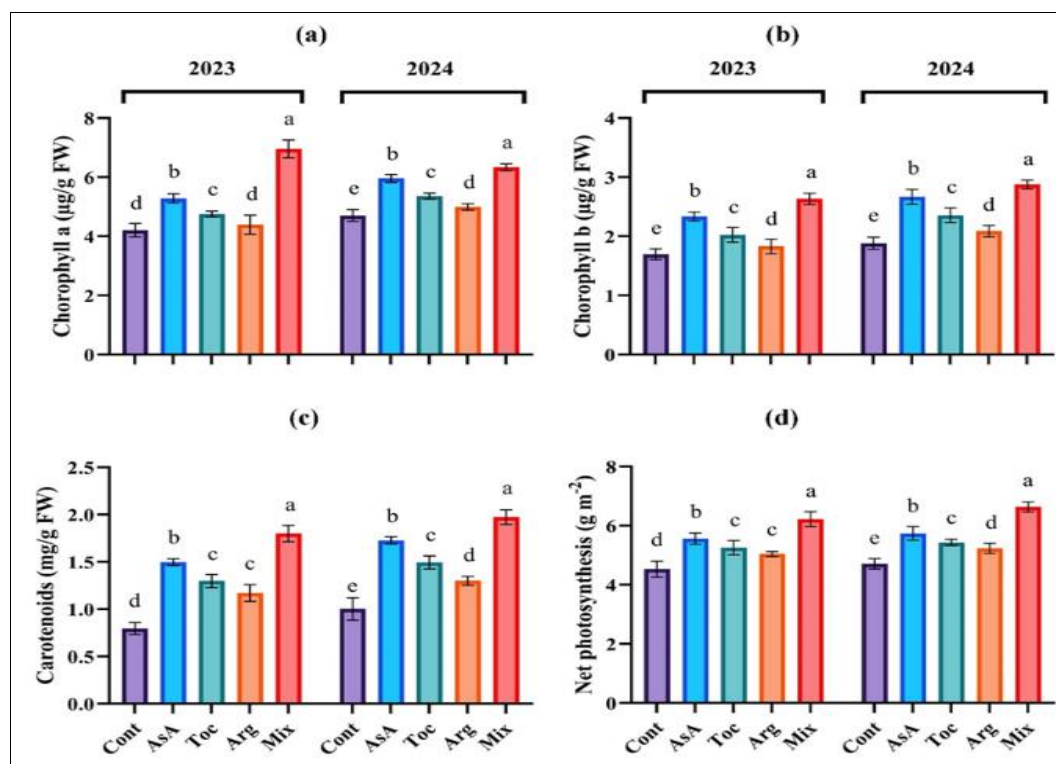


Figure 1. Effects of foliar application of ascorbic acid (AsA), tocopherol (Toc), arginine (Arg), and their combined application on chlorophyll a (a), chlorophyll b (b), carotenoid content (c), and net photosynthetic rate (d) of maize grown under naturally saline field conditions during the 2023 and 2024 growing seasons. Data are presented as mean $\pm$ SD ($n = 3$). Columns sharing the same letter are not significantly different according to Duncan's multiple range test at $P \leq 0.05$.

Stomatal conductance

Analysis of variance showed that foliar application of the tested biostimulants significantly improved stomatal conductance compared with the untreated control in both growing seasons (Figure 2a). The combined application of AsA, Toc and Arg consistently produced the highest values, exceeding the control by 52.3% and 62.7% in 2023 and 2024, respectively.

Among the individual treatments, AsA elicited the greatest response, followed by Toc, whereas Arg was the least effective, with only marginal improvement during the second season. Duncan's multiple range test further demonstrated that the combined treatment was significantly superior to all individual applications, highlighting a synergistic effect of the three biostimulants on stomatal regulation under naturally saline field conditions.

Transpiration rate

Analysis of variance revealed significant treatment effects on transpiration rate in both growing seasons. All biostimulant treatments significantly increased transpiration rate relative to the untreated control. The combined application of AsA, Toc and Arg consistently produced the greatest enhancement, increasing transpiration rate by 79.3% and 101.0% in the 2023 and 2024 growing seasons, respectively. Among the individual treatments, AsA was the most effective, with increases of 65.2% and 93.3%, followed by Toc (42.0% and 44.6%), whereas Arg showed the smallest response (24.5% and 23.6%). Duncan's multiple range test confirmed the significant superiority of the combined treatment over both the individual applications and the untreated control, indicating a synergistic effect of the three biostimulants on transpiration under naturally saline field conditions (Figure 2b).

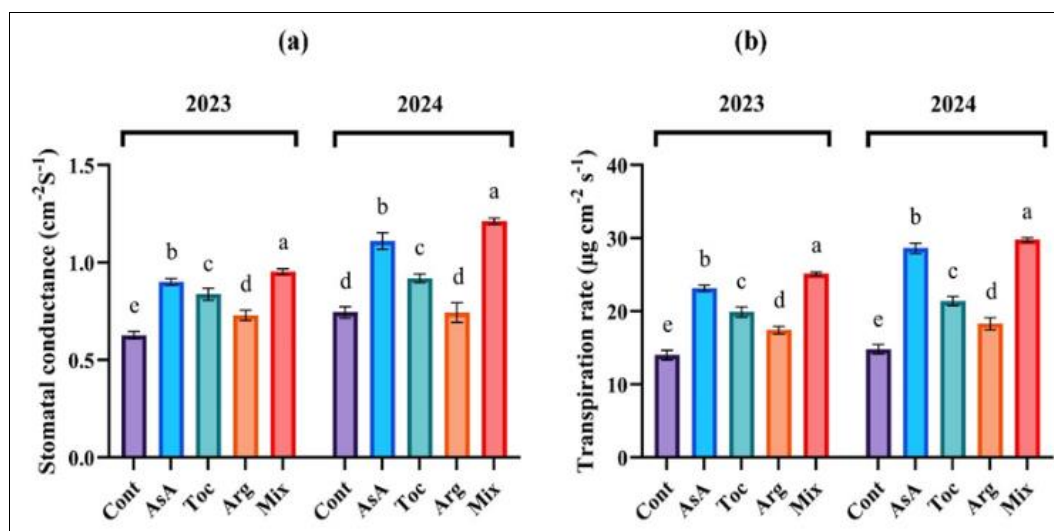


Figure 2. Effects of foliar application of ascorbic acid (AsA), tocopherol (Toc), arginine (Arg), and their combined application on stomatal conductance (a) and transpiration rate (b) of maize grown under naturally saline field conditions during the 2023 and 2024 growing seasons. Data are expressed as mean $\pm$ SD ($n = 3$). Different letters indicate significant differences among treatments according to Duncan's multiple range test at $P \leq 0.05$.

Proline content

Analysis of variance revealed significant treatment effects on proline accumulation in both growing seasons. All biostimulant treatments significantly increased proline content relative to the untreated control. The combined application of AsA, Toc and Arg produced the greatest response, increasing proline content by 65.5% and 76.2% during the 2023 and 2024 growing seasons, respectively. Among the individual treatments, Arg was the most effective, with increases of 45.8% and 47.2%, followed by AsA (36.3% and 37.9%), whereas Toc elicited the smallest, albeit significant, response (15.1% in both seasons). Duncan's multiple range test confirmed the significant superiority of the combined treatment over both the individual applications and the untreated control, indicating a synergistic effect of the three biostimulants on osmotic adjustment under naturally saline field conditions (Figure 3a).

Relative water content

Foliar application of the tested biostimulants significantly affected relative water content (RWC) in both growing seasons ($P \leq 0.05$; Figure 3b). Compared with the untreated control, all treatments improved leaf RWC, with the combined application of AsA, Toc and Arg consistently producing the highest values.

This treatment increased RWC by 42.8% and 56.9% in the 2023 and 2024 growing seasons, respectively. Among the individual applications, AsA showed the greatest effectiveness, followed by Toc, whereas Arg produced the smallest improvement. Duncan's multiple range test confirmed the significant superiority of the combined treatment over all individual applications and the untreated control, demonstrating a synergistic enhancement of plant water status under naturally saline field conditions (Figure 3b).

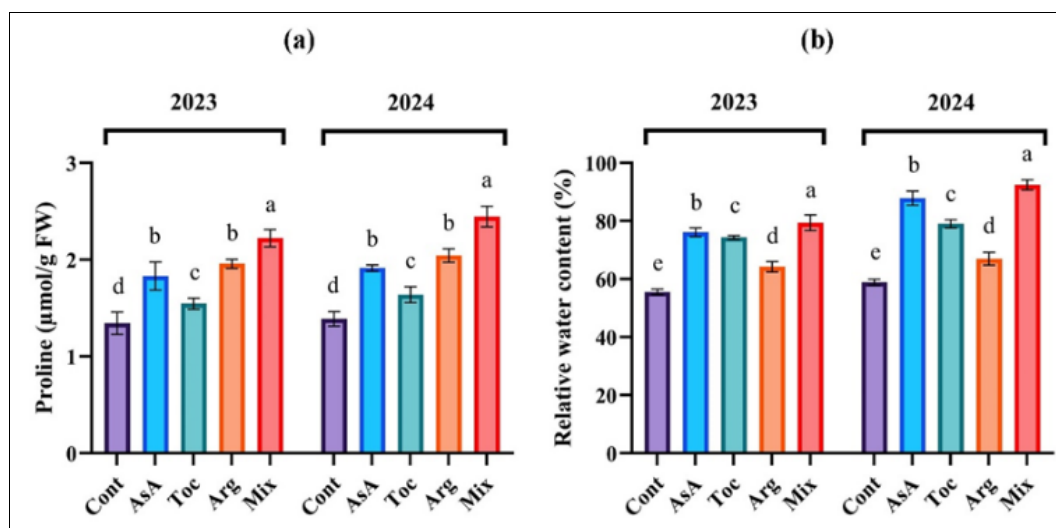


Figure 3. Effects of foliar application of ascorbic acid (AsA), tocopherol (Toc), arginine (Arg), and their combined application on proline content (a) and relative water content (b) of maize grown under naturally saline field conditions during the 2023 and 2024 growing seasons. Data are expressed as mean $\pm$ SD ($n = 3$). Means followed by the same letter are not significantly different according to Duncan's multiple range test at $P \leq 0.05$.

The soil salinity levels recorded during both growing seasons were sufficiently high to impose considerable salt stress on maize plants, exceeding the salinity threshold previously reported for this crop (Farooq et al., 2015; Acosta-Motos et al., 2017; El-Beltagi et al., 2025). The observed reductions in chlorophyll content, gas-exchange characteristics and relative water content (RWC) are consistent with the well-documented effects of salinity, which include oxidative damage, disruption of chloroplast structure, stomatal closure, impaired CO_2 assimilation and osmotic imbalance caused by excessive Na^+ and Cl^- accumulation (Akcin and Yalcin, 2016; Hameed et al., 2021; Shaheen et al., 2025; El-Beltagi et al., 2026). These physiological disturbances ultimately reduce photosynthetic efficiency and plant productivity, while the accumulation of compatible osmolytes such as proline represents an important adaptive mechanism that contributes to osmotic adjustment and cellular protection under saline conditions (Mahlooji et al., 2018; Boussora et al., 2024; El-Beltagi et al., 2024; Kaur et al., 2024).

Foliar application of AsA, Toc and Arg effectively alleviated the adverse effects of salinity by improving photosynthetic pigments, gas-exchange parameters, proline accumulation, and RWC. These improvements can be

attributed to their complementary physiological roles, including enhancement of antioxidant defense, stabilization of chloroplast and thylakoid membranes, protection against reactive oxygen species, maintenance of ion homeostasis, and promotion of osmotic adjustment (El-Kassas et al., 2020; Faizan et al., 2023; Hasanuzzaman et al., 2023; Sadaf et al., 2025). Similar findings have been reported in maize and other cereal crops under saline conditions (Seleiman et al., 2022).

Importantly, the marked increase in transpiration observed following biostimulant application should not be interpreted solely as an inherently beneficial response under saline conditions. Although enhanced transpiration may support CO_2 uptake and leaf cooling and was accompanied in the present study by higher stomatal conductance, net photosynthetic rate and leaf relative water content, greater water flux through the transpiration stream can also potentially increase the delivery of Na^+ to shoots if root-level ion exclusion and xylem Na^+ retrieval is insufficient (Shams and Khadivi, 2023; Zhang et al., 2023). Indeed, under salinity stress, stomatal conductance is closely associated with plant water use and evapotranspiration in maize, highlighting the importance of interpreting changes in transpiration together with stomatal regulation and carbon assimilation

(Liao et al., 2024). Thus, the physiological significance of the increased transpiration observed here should be considered in conjunction with plant water status, stomatal conductance, water-use efficiency and ionic homeostasis. In the present study, the combined AsA+Toc+Arg treatment increased transpiration more strongly than net photosynthesis, while simultaneously maintaining substantially higher RWC, suggesting that the enhanced water flux was associated with improved leaf hydration and gas-exchange performance rather than an isolated stimulation of water loss. However, water-use efficiency (WUE) represents the balance between carbon assimilation and transpiration, and therefore an increase in transpiration alone cannot be taken as evidence of improved WUE (Liang et al., 2023). Because WUE and tissue Na^+ and K^+ concentrations were not directly quantified in the present study, it cannot be concluded that the increased transpiration resulted in improved water-use efficiency or enhanced Na^+/K^+ homeostasis. Salt tolerance ultimately depends on the balance between water flux and the capacity of roots and vascular tissues to restrict Na^+ loading into the xylem, retrieve Na^+ from the transpiration stream, and maintain K^+ homeostasis (Shams and Khadivi, 2023; Zhang et al., 2023). Therefore, the beneficial physiological response observed in this study may reflect improved water relations and stomatal functioning, but the contribution of ion

exclusion and Na^+/K^+ homeostasis warrants direct investigation in future studies.

Among the individual treatments, AsA produced the greatest improvement in most physiological traits, reflecting its central role in protecting the photosynthetic apparatus and preserving chlorophyll biosynthesis (Conklin et al., 2024). However, the combined application of AsA, Toc, and Arg consistently resulted in the highest values for all measured parameters across both growing seasons, indicating a synergistic interaction among antioxidant protection, membrane stabilization, and osmotic regulation. The consistency of these responses over two successive seasons further demonstrates the robustness of the combined biostimulant strategy for enhancing maize tolerance to salinity under field conditions.

Figure 4 proposed schematic model illustrating the effects of foliar application of AsA, Toc and Arg, applied individually or in combination (1 g L^{-1}), on reactive oxygen species (H_2O_2 and $\text{O}_2^{\bullet-}$), malondialdehyde (MDA), membrane stability, chlorophyll a and b, net photosynthetic rate, proline accumulation, stomatal conductance, transpiration rate, and relative water content in maize grown under saline soil conditions. The schematic was created with the assistance of NotebookLM (Google) and subsequently reviewed, refined and validated by the authors to ensure scientific accuracy and consistency with the experimental findings.

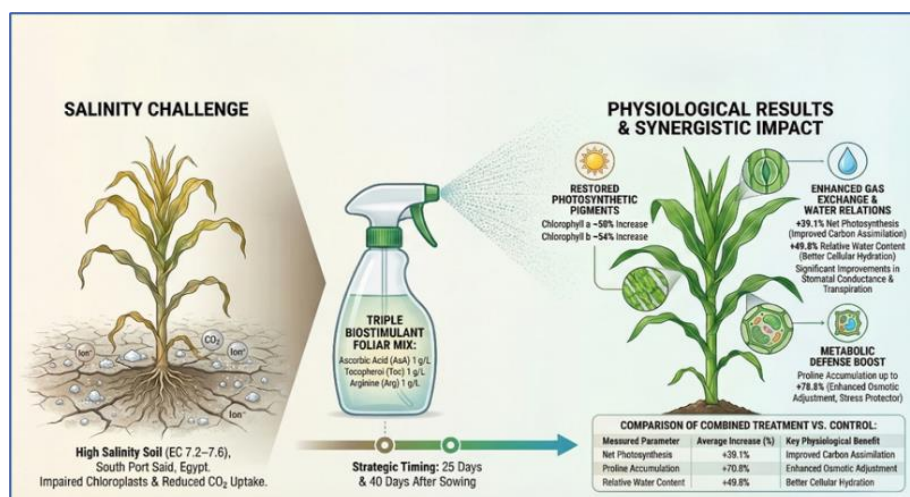


Figure 4. Proposed mechanism illustrating the role of foliar-applied ascorbic acid, tocopherol, and arginine in enhancing salinity tolerance through antioxidant defense, membrane stabilization, osmotic adjustment, and improvement of photosynthetic performance and water relations in maize.

CONCLUSIONS

This study demonstrated that foliar application of ascorbic acid (AsA), tocopherol (Toc), and arginine (Arg) effectively mitigated the adverse effects of salinity stress on maize. The combined application of AsA, Toc and Arg was the most effective treatment, enhancing photosynthetic performance, water status, and osmotic adjustment through improved antioxidant protection and membrane stability. These integrated physiological responses significantly increased salinity tolerance compared with individual applications. Therefore, the combined foliar application of AsA, Toc, and Arg represents a sustainable and eco-friendly strategy for improving maize performance under saline conditions, although further studies are needed to validate its effectiveness across diverse genotypes and environments.

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