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Salicylic and Ascorbic Acid Improve Growth, Yield, and Seed Quality of Sunflower under Prolonged Irrigation Intervals

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ABSTRACT

Climate change-driven freshwater scarcity has prompted the adoption of extended irrigation intervals to conserve water; however, this practice often compromises crop productivity and quality. This study evaluated the effectiveness of foliar-applied salicylic acid (SA) and ascorbic acid (AA), applied individually or in combination at 100 mM L^{-1}, in mitigating water deficit stress in sunflower grown under a prolonged irrigation interval (20 days) compared with the recommended 14-day interval during the 2024 and 2025 growing seasons. Extending the irrigation interval significantly reduced plant growth, leaf area index, photosynthetic pigments, nutrient accumulation (N, P, and K), seed yield, protein and oil contents, and the proportion of unsaturated fatty acids. In contrast, foliar application of SA or AA alleviated these adverse effects, while their combined application produced the greatest improvement. Treated plants exhibited enhanced vegetative growth, higher chlorophyll and carotenoid contents, higher seed N, P and K concentrations, superior seed yield and quality, and greater accumulation of unsaturated fatty acids. Moreover, these treatments improved seed yield and quality under the prolonged irrigation interval, demonstrating their potential to alleviate the adverse effects of water-deficit conditions. Overall, the combined application of SA and AA proved to be the most effective approach for enhancing drought tolerance, improving water use efficiency, and sustaining sunflower productivity under water-limited conditions.

Keywords: chlorophyll, saturated fatty acids, seed oil content, seed protein content, unsaturated fatty acids.

INTRODUCTION

Changing global climatic conditions, rising population growth, and the progressive decline in arable land have intensified concerns over global food security, emphasizing the urgent need to narrow the productivity gap between potential and actual yields (Mohamed et al., 2009; Anderson et al., 2020). Among the major factors Drought stress (DS) is one of the major abiotic constraints limiting crop productivity, particularly in regions characterized by increasing climatic variability and recurrent periods of water shortage. In sunflower, water deficit can markedly impair vegetative growth, leaf development, photosynthetic activity and yield formation (Kaleri et al., 2023). Previous studies have demonstrated that hydric stress substantially affects leaf area, chlorophyll content and other physiological traits in sunflower, while differences among genotypes indicate the existence of significant variation in drought response (Petcu et al., 2001). More recently, drought-induced yield reductions in sunflower have also been documented under Romanian agro-climatic conditions, highlighting the increasing vulnerability of this crop to water limitation (Brumă et al., 2025)

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Sunflower (*Helianthus annuus* L.) is a globally important oilseed crop due to its high-quality edible oil and adaptability to diverse environmental conditions. Cojocaru et al. (2023) emphasized that climatic conditions can substantially influence sunflower oil content and fatty acid composition. Despite its moderate tolerance to water deficit, sunflower productivity remains highly dependent on adequate water availability, making drought a major threat to sustainable production systems (Keipp et al., 2020; Abdel-Fattah et al., 2023). Drought stress disrupts photosynthesis through chlorophyll degradation, stomatal closure and overproduction of ROS (reactive oxygen species), thereby causing oxidative damage, impaired nutrient uptake, lower biomass production, and significant yield losses (Ghaffari et al., 2023; Janzen et al., 2023). Moreover, reproductive stages are particularly sensitive to water limitation, negatively affecting seed development, oil accumulation, and seed quality.

Exogenous application of plant growth regulators and antioxidants has been identified as an effective approach to mitigate drought-induced damage. Salicylic acid (SA), a significant signaling molecule, enhances antioxidant enzyme activities, regulates stomatal behavior, and promotes osmotic adjustment during stress conditions (Pandey et al., 2015; El-Beltagi et al., 2025a, b). Similarly, ascorbic acid (AA), a major non-enzymatic antioxidant, scavenges ROS, protects chloroplast structures, stabilizes cellular membranes, and maintains photosynthetic efficiency during water deficit (WD) (Abdul Khaliq et al., 2026). Prior research has indicated that SA and AA improve nutrient acquisition, antioxidant capacity and stress tolerance in several crop species (El-Bially et al., 2022; Saudy et al., 2023; El-Beltagi et al., 2026).

Increasing evidence suggests that the co-treatment of SA and AA may provide synergistic effects by enhancing antioxidant defense systems (AODSs), preserving membrane integrity, improving chlorophyll content, and reducing oxidative damage (OD) more effectively than individual treatments (Abdul Khaliq et al., 2026). However, information regarding the effectiveness of their combined foliar application in sunflower under drought conditions (DC) remains insufficiently studied.

The objective of the present study was to evaluate the effects of foliar spraying of salicylic acid and ascorbic acid, alone and in combination, on alleviating drought-induced stress and improving physiological traits, nutrient status, growth performance, yield attributes, and seed quality of sunflower plants grown under conditions of water deficit.

MATERIAL AND METHODS

Experimental site, treatments and study design

A field experiment was carried out during the 2024 and 2025 summer growing seasons at the Sakha Agricultural Research Station, Kafr El-Sheikh, Egypt (31°07′N, 30°05′E). The study evaluated the effects of foliar application of SA and AA at 100 mM L^{-1}, used singly or in combination, on sunflower plants subjected to an extended irrigation interval of 20 days, compared with the recommended irrigation interval of 14 days. Foliar treatments were sprayed until complete leaf coverage without causing runoff and were applied twice during the growing period: at the stem elongation stage (25 days post-sowing) and at the flowering stage (50 days after sowing). The experiment was arranged as a **2 × 4 factorial experiment** in a randomized complete block design (RCBD) with four replications. The first factor was the irrigation interval, consisting of two levels: the recommended 14-day irrigation interval and the prolonged 20-day irrigation interval. The second factor was foliar application, consisting of four levels: untreated control, salicylic acid (SA), ascorbic acid (AA), and the combined SA + AA treatment. Thus, the experiment comprised eight treatment combinations, with each combination represented by four replicates. The same experimental design was followed during both the 2024 and 2025 growing seasons.

Field preparation and crop management practices

Following the harvest of the preceding wheat crop, the experimental field was prepared using standard tillage practices, including two ploughings followed by harrowing and ridging at 0.70 m spacing. The field was subsequently divided into plots measuring 42 m² (6 m × 7 m), each consisting of 10 rows, 6 m in length and 0.70 m in width. To minimize potential interference from irrigation water movement and spray drift among treatments, buffer ditches 1.5 m wide were established between adjacent plots.

Sunflower seeds were sown manually in hills spaced 25 cm apart on one side of each ridge. Seedlings were thinned to one plant per hill before the first irrigation, which was applied 21 days after sowing. In the land preparation stage, phosphorus fertilizer was incorporated at a dosage of 40 kg P_2O_5 ha^{-1} as calcium superphosphate (15.5% P_2O_5). Nitrogen fertilizer was applied as urea at the recommended rate of 60 kg N per feddan. Sowing was conducted on 15 and 18 May, while harvesting took place on 2 and 6 September during the 2024 and 2025 growing seasons, respectively. All other crop management practices, were implemented according to the recommendations of the Agricultural Research Center.

Plant growth traits

Plant height was determined at harvest by recording plant height from the soil surface to the apical point of the plant using a ruler or measuring tape. Measurements were recorded from ten randomly selected plants per plot, and the average value was calculated. Leaf area was measured using a Laser Area Meter (CI-203, CID Bio-Science, Camas, WA, USA). The leaf area index (LAI) was subsequently derived from the ratio of total leaf area to the corresponding ground surface area as described by Gardner et al. (1985).

Chlorophyll and carotenoids content

Chlorophyll *a*, *b*, and total carotenoids, were quantified using fresh leaf samples taken from the third fully mature leaf counted from the apex at 70 days after germination. Fresh leaf tissue (0.5 g) was homogenized in 50 mL of acetone using a tissue homogenizer, followed by centrifugation at 10,000 rpm for 15 min at 4°C. The resulting extract was filtered through a 0.45 µm nylon syringe filter, and the clear supernatant was used for pigment analysis.

The contents of chl. *a*, *b*, and carotenoids were assessed spectrophotometrically using a Spectronic 20 Genesys spectrophotometer (Thermo Scientific, USA). Absorbance readings were assessed at 645 and 655 nm for chl. *a* and *b*, respectively, whereas carotenoid content was measured at 480 and 495 nm. All analyses were conducted in triplicate, and pigment content were quantified using the method described by Bulda et al. (2008).

Seed nutrient content

Dried sunflower seed samples were digested using wet digestion with a sulfuric-perchloric acid mixture prior to nutrient analysis. Nitrogen (N) concentration was determined using the Kjeldahl method, conversely phosphorus (P) and potassium (K) contents were measured spectrophotometrically and by flame photometry, respectively, as described by Walinga et al. (2013).

Yield and its components

Seed number/plant was determined by manually counting all filled seeds obtained from each plant after threshing, while seed weight per plant was recorded by weighing the total seed number per plant, determined using a precision balance with 0.01 g accuracy. For seed yield per plot, all seeds were harvested from each experimental plot, dried to a constant moisture content of approximately 10-12%, and weighed in kilograms per plot. To enable comparison among treatments, plot yield values were subsequently converted to seed yield per hectare (t ha^{-1}) using the appropriate area conversion factor.

Seed protein content

Crude protein content in the seeds was assessed using the Macro-Kjeldahl method according to the procedures outlined by the Association of Official Analytical Chemists (AOAC, 2000). Total crude protein concentration was calculated by multiplying the total nitrogen content by a conversion factor of 6.25.

Seed oil content

Seed samples were oven-dried at 60°C to a stable weight and subsequently ground into a fine powder. Oil extraction was performed using 30 g of the dried seed powder with petroleum ether (boiling point range: 40-60°C) in a Soxhlet extraction apparatus for 6-8 h. Following extraction, the solvent was eliminated under reduced pressure, and the extracted oil was weighed to determine oil content, following the method described by Mostafa and Afify (2022).

Saturated unsaturated fatty acids content

Sunflower seeds were oven-dried (60°C), ground, and subjected to Soxhlet extraction with petroleum ether (40-60°C). Oil content was determined gravimetrically according to Association of Official Analytical Chemists Method 920.39 (AOAC, 2016). Fatty acid methyl esters (FAMEs) were prepared by methanolic KOH transesterification (Christie, 1982) and analyzed by GC-FID using an SP-2560 capillary column. Fatty acids were identified by comparison with a Supelco 37 Component FAME standard, and results were expressed as the percentage of total identified fatty acids.

Statistical Analysis

The collected data were subjected to two-way analysis of variance (ANOVA) appropriate for the 2 × 4 factorial experimental design, considering irrigation interval, foliar treatment, and their interaction as sources of variation. The analyses were conducted separately for each growing season. Treatment effects were considered statistically significant at $p \leq 0.05$. When significant differences were detected, treatment means were compared using Duncan's multiple range test at the 5% probability level. All statistical analyses were performed using COSTAT statistical software (CoHort Software, 1986). Following the detection of significant differences, treatment means were compared via Duncan's multiple range test (Duncan, 1955).

RESULTS AND DISCUSSION

Plant height and leaf area index

As shown in Figure 1, extending the irrigation interval to 20 days significantly reduced plant height and leaf area index (LAI) compared with the 14-day interval in both seasons. Plant height declined by 24.0% and 27.0%, while LAI decreased by 31.3% and 32.2% in 2024 and 2025, respectively. Foliar application of SA and AA individually or combined, significantly improved both traits under water deficit. The combined SA + AA treatment was the most effective, increasing plant height by 27.4% and 34.3% and LAI by 40.7% and 35.4% relative to drought-stressed controls in 2024 and 2025, respectively. Moreover, plant height under the combined treatment was comparable to that of untreated plants under optimal irrigation, demonstrating that the combined application of SA and AA effectively mitigated the adverse effects of water deficit on sunflower vegetative growth.

Chlorophyll a, chlorophyll b and carotenoids content

Figure 2 shows that extending the irrigation interval to 20 days significantly reduced chlorophyll a, chlorophyll b, and carotenoid contents compared with the 14-day irrigation regime in both seasons. Chlorophyll a declined by 49.4% and 37.7%, chlorophyll b by 53.9% and 47.5%, and carotenoids by 34.4% and 36.6% in 2024 and 2025, respectively. Foliar application of SA and AA individually or combined, significantly increased photosynthetic pigment contents under drought stress, with no significant differences between the individual treatments. The combined SA + AA treatment was the most effective, increasing chlorophyll a by 85.5% and 48.2%, chlorophyll b by 107.1% and 79.2%, and carotenoids by 49.2% and 51.9% relative to drought-stressed controls in 2024 and 2025, respectively. These findings indicate that the combined application of SA and AA effectively alleviated drought-induced pigment degradation and preserved the photosynthetic apparatus under water-deficit conditions.

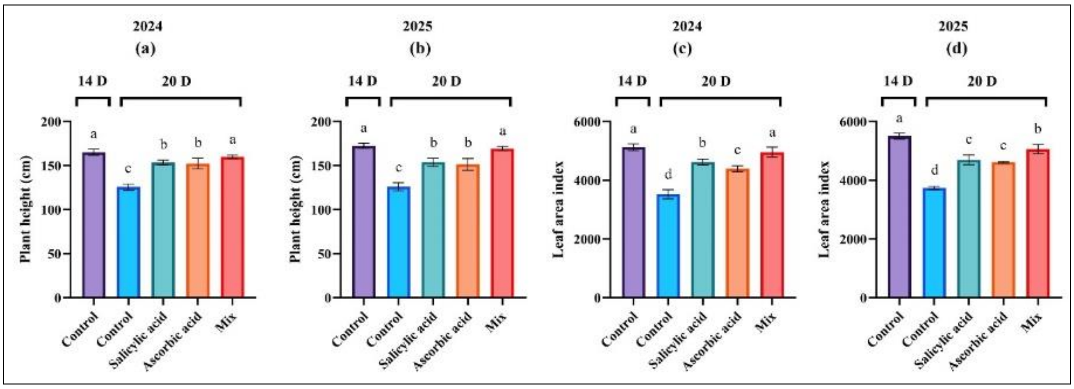

Figure 1. Responses of plant height (a, b) and leaf area index (c, d) of sunflower to foliar application of salicylic acid and ascorbic acid at 100 mM, applied individually or as a combined treatment (Mix), under 14- and 20-day irrigation intervals during the 2024 and 2025 growing seasons. Data are given as mean ± SD. Bars sharing different letters demonstrate significant differences following Duncan's multiple range test ($P \leq 0.05$).

Seed nutrient content

Figure 3 shows that extending the irrigation interval to 20 days significantly decreased seed N, P, and K contents compared with the 14-day irrigation regime in both seasons. Seed N declined by 37.3% and 41.4%, P by 45.9% and 40.2%, and K by 44.4% and 32.6% in 2024 and 2025, respectively. Foliar application of salicylic acid (SA) and ascorbic acid (AA), individually or combined, significantly improved seed nutrient concentrations under drought stress, with no significant differences between the individual treatments. The combined SA + AA treatment was the most effective, increasing seed N by 53.6% and 64.4%, P by 70.7% and 54.8%, and K by 66.8% and 38.4% relative to drought-stressed controls in 2024 and 2025, respectively. These results demonstrate that the combined application of SA and AA alleviated drought-induced reductions in seed N, P, and K concentrations, thereby improving the nutritional quality of sunflower seeds under water-deficit conditions.

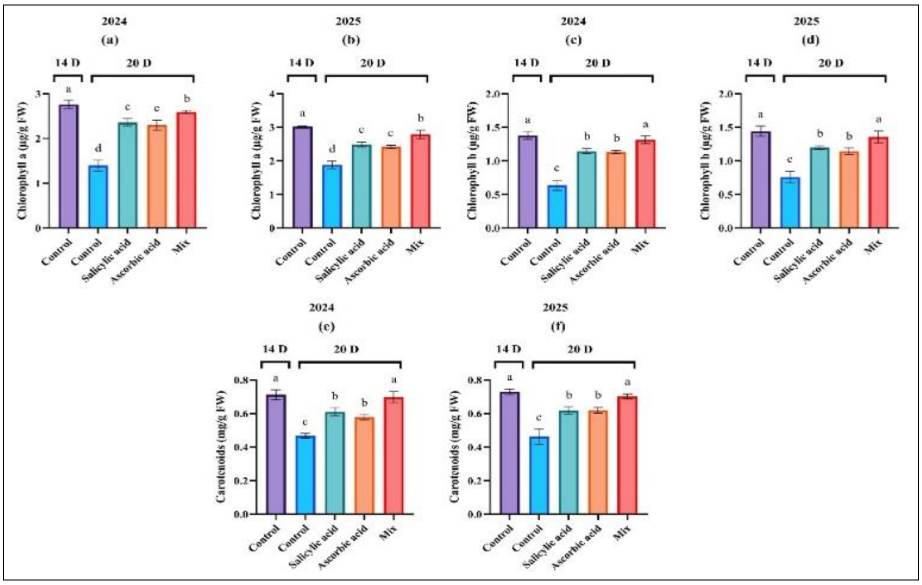

Figure 2. Responses of chl. *a* (a, b), chl. *b* (c, d) and carotenoids (e, f) content of sunflower to foliar application of ascorbic acid (AA) and salicylic acid (SA) at 100 mM, applied individually or as a combined treatment (Mix), under 14- and 20-day irrigation intervals in the 2024 and 2025 seasons. Values are reported as mean ± standard deviation (SD). Means represented by bars bearing different letters differ significantly based on Duncan's multiple range test ($P \leq 0.05$).

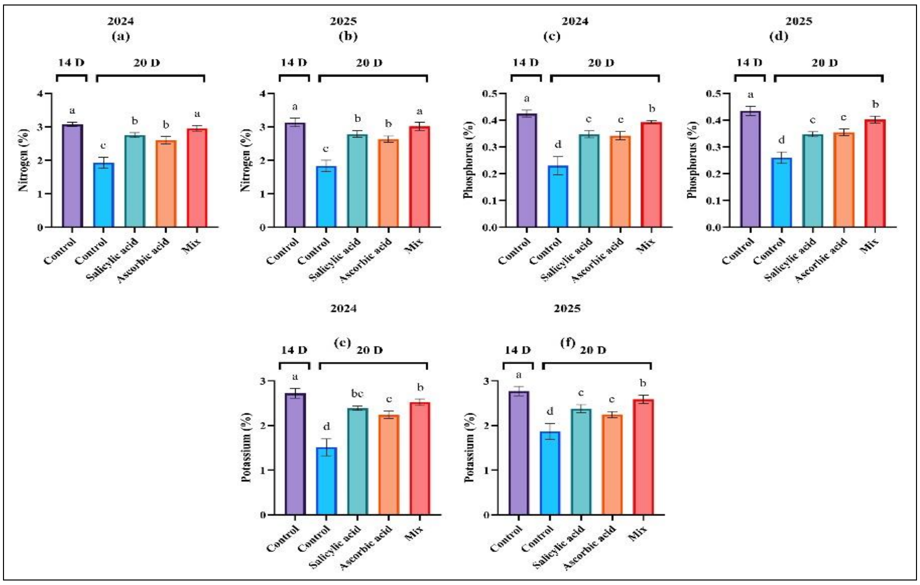

Figure 3. Responses of seed nutrient concentrations including N% (a, b), P% (c, d) and K% (e, f) of sunflower to foliar application of ascorbic acid (AA) and salicylic acid (SA) at 100 mM, applied individually or as a combined treatment (Mix), under 14- and 20-day irrigation intervals during the 2024 and 2025 growing seasons. Data are expressed as mean ± SD. Different letters above bars indicate significant differences based on Duncan's multiple range test ($P \leq 0.05$).

Yield and yield components

Figure 4 shows that extending the irrigation interval to 20 days significantly reduced seed number, seed weight per plant, and seed yield compared with the optimal 14-day irrigation regime in both growing seasons. Seed number declined by 28.0% and 31.4%, seed weight per plant by 38.0% and 36.6%, and seed yield by 37.1% and 35.0% in 2024 and 2025, respectively. Foliar application of SA and AA, individually or combined, significantly improved yield and its components under drought stress, with no significant differences between the individual treatments for most traits. The combined SA + AA treatment was the most effective, increasing seed number by 34.8% and 39.2%, seed weight per plant by 57.5% and 48.0%, and seed yield by 50.2% and 43.5% relative to drought-stressed controls in 2024 and 2025, respectively. These results demonstrate that the combined application of SA and AA effectively mitigated the adverse effects of water deficit on reproductive development and sunflower productivity.

Seed protein content

Figure 5 shows that extending the irrigation interval to 20 days significantly reduced seed protein content by 23.3% and 24.9% in the 2024 and 2025 growing seasons, respectively, compared with the optimal 14-day irrigation regime. Foliar application of salicylic acid (SA) and ascorbic acid (AA), either individually or in combination, significantly enhanced seed protein content under drought stress. Although SA produced a greater increase than AA, the difference between the two individual treatments was not statistically significant. Relative to drought-stressed plants, SA increased seed protein content by 18.7% and 19.9%, whereas AA increased it by 14.8% and 15.5% in 2024 and 2025, respectively.

Notably, the combined treatment of SA and AA exerted the most pronounced effect, outperforming the individual treatments. Relative to drought-stressed plants receiving no treatment, the combined treatment increased seed protein content by 30.1% and 29.6% in the 2024 and 2025 seasons, respectively. These results suggest a synergistic effect of SA and AA in mitigating drought-induced reductions in seed protein accumulation.

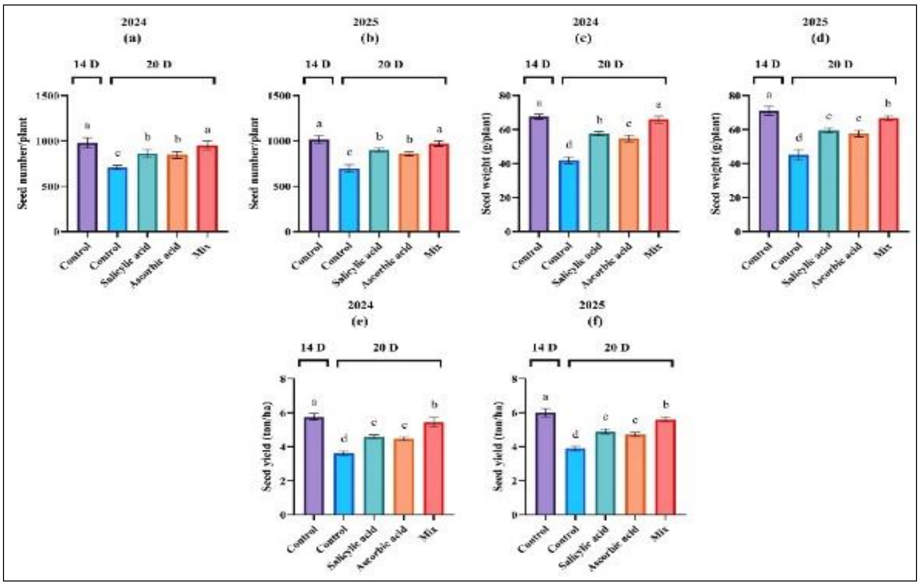

Figure 4. Responses of yield and its components including number of seed/plant (a, b), seed weight/plant (c, d) and seed yield (ton/ha) (e, f) of sunflower to foliar application of ascorbic acid (AA) and salicylic acid (SA) at 100 mM, applied individually or as a combined treatment (Mix), under 14- and 20-day irrigation intervals during the 2024 and 2025 growing seasons. Values are reported as mean ± SD. Bars with different letters are significantly different based on Duncan's multiple range test ($P \leq 0.05$).

Oil, saturated and unsaturated fatty acids content

Figure 6 shows that extending the irrigation interval to 20 days significantly reduced seed oil content and unsaturated fatty acids while increasing saturated fatty acids compared with the optimal 14-day irrigation regime in both growing seasons. Seed oil content declined by 21.0% and 20.6%, and unsaturated fatty acids by 15.4% and 18.2%, whereas saturated fatty acids increased by 29.3% and 31.4% in 2024 and 2025, respectively. Foliar application of salicylic acid (SA) and ascorbic acid (AA), individually or combined, significantly improved seed oil quality under drought stress. The combined SA + AA treatment was the most effective, increasing seed oil content by 20.5% and 18.7% and unsaturated fatty acids by 22.4% and 18.6%, while reducing saturated fatty acids by 21.9% and 21.4% relative to drought-stressed controls in 2024 and 2025, respectively. These findings demonstrate that the combined application of SA and AA effectively mitigated drought-induced deterioration in seed oil quantity and quality by promoting unsaturated fatty acid accumulation and limiting saturated fatty acid content.

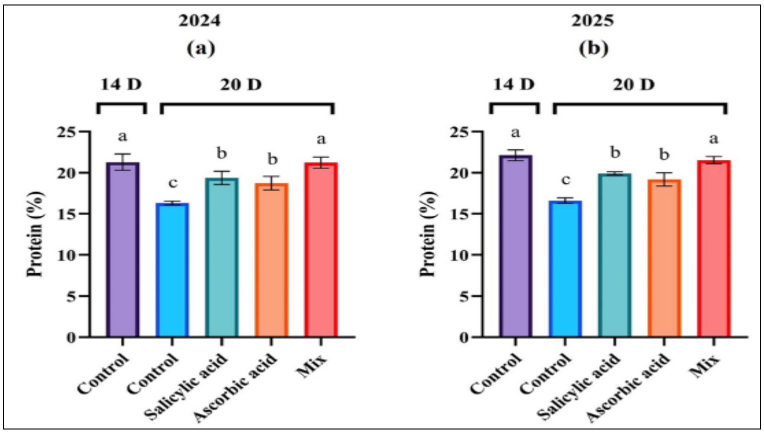

Figure 5. Responses of protein content% (a, b) of sunflower to foliar use of ascorbic acid) and salicylic acid at 100 mM, applied individually or as a combined treatment (Mix), under 14- and 20-day irrigation intervals during the 2024 and 2025 growing seasons. Data are expressed as mean ± SD. Bars annotated with different letters are significantly different as determined by Duncan's multiple range test ($P \leq 0.05$).

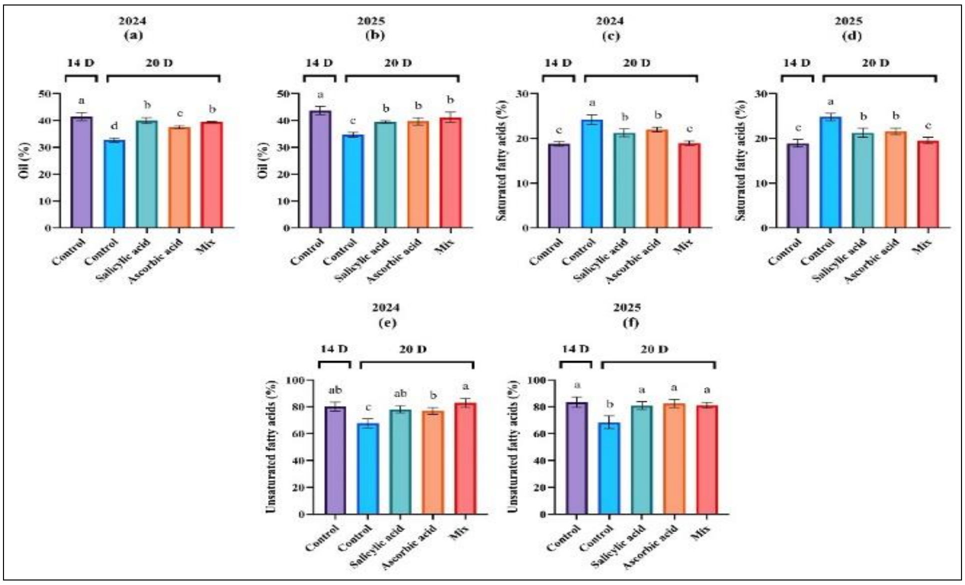

Figure 6. Responses of oil% (a, b), saturated fatty acids (c, d) and unsaturated fatty acids (e, f) of sunflower to foliar application of ascorbic acid (AA) and salicylic acid (SA) at 100 mM, applied individually or as a combined treatment (Mix), under 14- and 20-day irrigation intervals during the 2024 and 2025 growing seasons. Data are expressed as mean ± SD. Means represented by bars bearing different letters differ significantly based on Duncan's multiple range test ($P \leq 0.05$).

Extending the irrigation interval to 20 days markedly impaired sunflower growth, photosynthetic performance, seed nutritional quality, oil composition, and yield. These reductions are mainly attributable to drought-induced disruption of plant water status, which restricts cell division and elongation, suppresses leaf expansion, accelerates chlorophyll degradation, and reduces photosynthetic carbon assimilation (Yang et al., 2021; Bijalwan et al., 2025; Karami et al., 2025). The decline in carotenoid concentration further indicates weakened photoprotection against drought-induced oxidative stress, while reduced root activity and nutrient transport under water deficit limited N, P, and K accumulation, resulting in lower seed protein content (Ghaffari and Hoseinlou, 2013; Yang et al., 2021). Moreover, impaired carbon allocation and altered fatty acid metabolism decreased seed oil content and unsaturated fatty acids, thereby reducing oil quality (Ashraf and Siddiqi, 2024; Bijalwan et al., 2025).

Foliar application of salicylic acid (SA) and ascorbic acid (AA), individually and particularly in combination, substantially alleviated the detrimental effects of drought. The observed improvements in growth, photosynthetic pigments, seed N, P, and K concentrations, seed protein, oil quality, and yield are associated with enhanced antioxidant defense, improved osmotic adjustment, greater membrane stability, and sustained photosynthetic efficiency under water-deficit conditions (Damalas and Koutroubas, 2022; El-Hendawy et al., 2024; Abdul Khaliq et al., 2026). SA promotes antioxidant enzyme activities, osmolyte accumulation, and stomatal regulation, whereas AA directly scavenges reactive oxygen species and protects chloroplast membranes from oxidative damage, thereby preserving chlorophyll, carotenoids, and carbon assimilation (Abdul Khaliq et al., 2026). The higher seed N, P, and K concentrations observed following foliar application of SA and AA may have contributed to the improved nutritional quality of sunflower seeds under drought conditions. However, because total nutrient uptake and translocation were not directly quantified in the present study, these responses are interpreted specifically in terms of changes in seed nutrient concentrations.

The superiority of the combined SA + AA treatment demonstrates a synergistic interaction between the two biostimulants. Their complementary mechanisms strengthen enzymatic and non-enzymatic antioxidant defenses, improve osmotic regulation, preserve membrane integrity, maintain photosynthetic activity, and enhance nutrient acquisition more effectively than either compound alone (Hayat et al., 2010; Akram et al., 2017). Consequently, reproductive development was better sustained, resulting in higher seed number, seed weight, seed yield, and improved oil quality under prolonged irrigation intervals. Collectively, these findings indicate that the combined application of SA and AA represents an effective strategy for improving drought tolerance, maintaining seed quality, and sustaining sunflower productivity under prolonged irrigation intervals. However, because the actual volume of irrigation water applied was not quantified in the present study, conclusions regarding water consumption or water productivity should be avoided.

As illustrated in Figure 7, foliar application of SA and AA at 100 mM, either individually or in combination, alleviated several of the adverse effects associated with the prolonged 20-day irrigation interval compared with the 14-day interval. The measured responses included improvements in plant height, LAI, photosynthetic pigment concentrations, seed N, P and K concentrations, seed protein and oil contents, fatty acid composition, and yield-related traits. The figure also presents several physiological mechanisms proposed in previous studies to explain the beneficial effects of SA and AA under water-deficit conditions; however, these mechanisms were not directly measured in the present experiment.

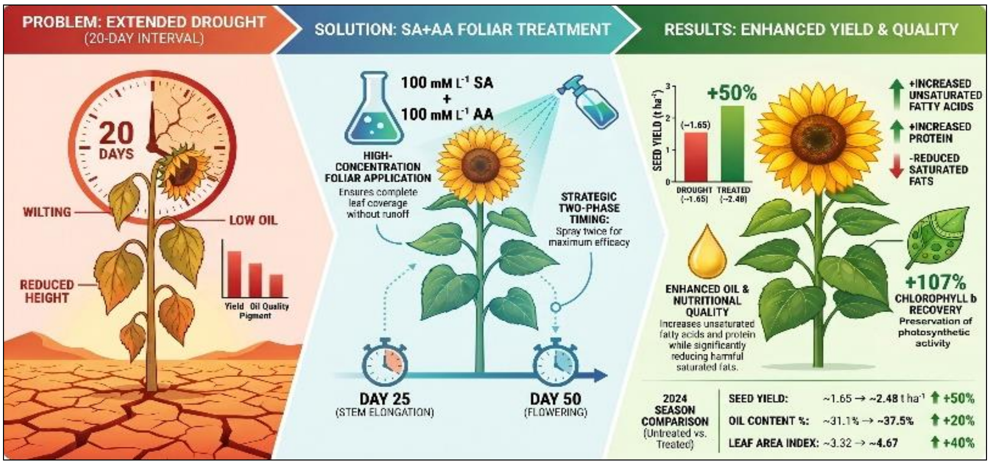

Figure 7. Schematic representation of the effects of foliar application of salicylic acid (SA) and ascorbic acid (AA) at 100 mM, applied individually or in combination at 25 and 50 days after sowing, on sunflower plants subjected to a prolonged irrigation interval of 20 days compared with the optimal 14-day interval. The figure distinguishes between measured outcomes of the present study and proposed physiological mechanisms based on previous literature. Measured outcomes include plant growth, photosynthetic pigment contents, seed N, P and K concentrations, yield and its components, seed protein and oil contents, and fatty acid composition. Proposed mechanisms, including enhanced antioxidant defense, improved osmotic regulation, membrane stabilization, stomatal regulation, and preservation of photosynthetic activity, are presented as literature-supported interpretations and were not directly measured in the present study.

CONCLUSIONS

Extending the irrigation interval to 20 days significantly reduced sunflower growth, photosynthetic pigment content, nutrient accumulation, seed quality, and yield, highlighting the detrimental effects of drought stress on plant physiological and metabolic processes. Foliar application of salicylic acid (SA) and ascorbic acid (AA), particularly in combination, effectively alleviated these adverse effects by enhancing antioxidant defense, preserving photosynthetic activity, increasing seed N, P, and K concentrations, and maintaining osmotic balance. Consequently, treated plants exhibited higher growth, yield components, seed yield, protein and oil contents, and improved fatty acid composition under water-deficit conditions. The superior performance of the combined SA + AA treatment demonstrates a synergistic interaction between the two biostimulants, making their combined foliar application a promising, sustainable, and environmentally friendly strategy for improving sunflower productivity and seed quality under deficit irrigation.

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