

Article

Foliar Selenium Application During Flowering and Fruiting Alleviates Drought-Induced Oxidative Damage and Promotes Tomato Growth

Haixue Cui ^{1,2,3}, Yuan Zhong ^{2,3}, Huanhuan Li ^{2,3}, Xiaoman Qiang ^{2,3}, Lijian Sun ⁴, Fukui Gao ^{2,3}, Gang Wang ^{1,*} and Hao Liu ^{2,3,*} 

¹ College of Water Conservancy and Civil Engineering, Shandong Agricultural University, Tai'an 271018, China; cuihaixue0115@163.com

² Key Laboratory of Crop Water Use and Regulation, Ministry of Agriculture and Rural Affairs, Farmland Irrigation Research Institute, Chinese Academy of Agricultural Sciences, Xinxiang 453002, China; 15008276676@163.com (Y.Z.); lihuanhuan@caas.cn (H.L.); qiangxiaoman@caas.cn (X.Q.); gaofukui1207@163.com (F.G.)

³ Shangqiu Agro-Ecological System National Observation and Research Station, Shangqiu 476000, China

⁴ Henan Provincial People's Victory Canal Support Center, Xinxiang 453000, China; slj93699@163.com

* Correspondence: gwang@sdaa.edu.cn (G.W.); liuhao03@caas.cn (H.L.)

Abstract

Drought stress induced by climate change is a major limiting factor for crop growth. Selenium (Se) is recognized as an important exogenous regulator that can mitigate drought and other abiotic stresses, but the effects of Se application at different growth stages remain unclear. In this study, greenhouse-grown tomato plants were subjected to four Se treatments (T1: control; T2: Se at seedling stage; T3: Se at flowering stage; T4: Se at both stages) combined with three irrigation regimes (W1: 50–55%, W2: 65–70%, W3: 80–85% of field capacity). The impacts of Se timing on antioxidant enzymes, osmotic regulators, and growth parameters were evaluated. Drought stress induced oxidative damage, reduced photosynthesis, and inhibited biomass accumulation, while proline content increased with drought severity. Se application showed clear growth-stage specificity: under mild stress, Se at the flowering stage most effectively enhanced antioxidant activity, regulated proline metabolism, improved photosynthetic performance, and promoted growth. Dual-stage application did not provide additional benefits. These findings indicate that applying Se during the flowering and fruiting stage is optimal for alleviating drought-induced growth inhibition in tomato. The results contribute to understanding Se-mediated drought tolerance and may support the development of stage-specific Se fertilizer management strategies.

Keywords: antioxidant system; drought stress; enzymatic reaction; exogenous selenium; greenhouse tomato; photosynthesis



Academic Editors: Jorge González-Villagra and Marjorie Reyes-Díaz

Received: 3 September 2025

Revised: 7 October 2025

Accepted: 11 October 2025

Published: 14 October 2025

Citation: Cui, H.; Zhong, Y.; Li, H.; Qiang, X.; Sun, L.; Gao, F.; Wang, G.; Liu, H. Foliar Selenium Application During Flowering and Fruiting Alleviates Drought-Induced Oxidative Damage and Promotes Tomato Growth. *Horticulturae* **2025**, *11*, 1242. <https://doi.org/10.3390/horticulturae11101242>

Copyright: © 2025 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>).

1. Introduction

Tomato (*Solanum lycopersicum* L.) is one of the most important vegetable crops globally, with a total cultivated area of 4.9 million hectares and an annual production of 186.1 million tons. As the largest producer, China contributes nearly one-quarter of global tomato output [1,2]. As a dicotyledonous plant, tomatoes are rich in lycopene, a compound proven to enhance the skin's resistance to harmful ultraviolet radiation and reduce mortality from various chronic diseases [2]. In recent years, the development of protected

cultivation techniques such as greenhouses, smart irrigation, and precision fertilization has significantly advanced the off-season tomato industry [3,4]. These technologies not only ensure vegetable supply in regions unsuitable for open-field cultivation but also drive local agricultural economies, further strengthening China's strategic position in the global tomato industry [5].

The rapid pace of global climate change has led to more frequent occurrences of extreme weather events [6]. Drought stress has become one of the most severe abiotic stress factors limiting crop growth and development. Under drought conditions, plants produce excessive reactive oxygen species (ROS), which have strong oxidative potential and cause serious oxidative damage to cellular structures. To cope with such stresses, plants activate a variety of defense mechanisms [7,8], among which the activation of antioxidant enzymes plays a key role in scavenging excess ROS and enhancing drought tolerance. However, the endogenous compounds produced by plants are often insufficient to counteract prolonged or severe drought stress [9], making the application of exogenous regulators an effective strategy to enhance stress resilience.

In response to the negative impacts of drought and other abiotic stresses on crop production, various exogenous regulators have been developed. Elements such as silicon and selenium have been applied to enhance crop drought tolerance [10]. Se, an essential micronutrient for humans, can boost immune function and provide anti-cancer and anti-aging effects when consumed in moderation [11]. However, direct dietary supplementation with Se can lead to toxicity. Plants play a pivotal role in the Se ecological cycle, making Se-enriched crops a safe and efficient method for human Se supplementation. In fact, plants are the only direct dietary source of Se for humans and animals [12]. Therefore, foliar application of Se to crops holds both agronomic and public health significance.

The application of exogenous selenium has been confirmed to significantly enhance the overall tolerance of plants to abiotic stresses. Studies have shown that foliar selenium application during the seedling stage not only promotes plant growth traits but also effectively activates the antioxidant defense system while simultaneously increasing the total phenolic compounds [13,14]. These physiological responses suggest that selenium application at the seedling stage can establish a fundamental antioxidant defense system, thereby laying the physiological foundation for subsequent stress responses. During the reproductive growth stage, selenium application at the flowering and fruit-setting stage exhibits more pronounced stress-mitigating effects. This treatment significantly reduces the accumulation of H_2O_2 and $O_2^{\cdot-}$ in leaves and, through the synergistic upregulation of osmotic regulatory substances and antioxidant enzyme activities, effectively alleviates drought-induced negative effects such as membrane lipid peroxidation. Consequently, it maintains the preferential allocation of photosynthetic products, ensuring the development of reproductive organs and yield formation [15,16]. In addition, selenium also exerts protective effects at the level of cellular ultrastructure. Under drought stress, selenium treatment helps maintain the integrity of chloroplast structures, inhibits thylakoid membrane degradation, and enhances chlorophyll stability [17]. These structural protective effects are further translated into improvements in photosynthetic performance [18]. However, some studies have reported that exogenous selenium application does not significantly affect stomatal density and size under drought stress [19]. Other studies have indicated that high concentrations of selenium may even decrease leaf water potential, leading to reduced transpiration rate and stomatal conductance, thereby partially limiting water transport efficiency. This suggests that the stomatal regulatory effects of selenium may depend on both concentration and species [20].

In summary, whether selenium is applied during the seedling stage or the flowering and fruit-setting stage, its accumulation in plants is regulated by factors such as application

concentration, method of application, and growth stage characteristics [21]. To optimize selenium application strategies across different growth stages, the present study investigated the effects of exogenous selenium application in tomato at different developmental stages (single application at the seedling stage, single application at the flowering stage, and dual application at both seedling and flowering stages). The study evaluated how selenium application mitigates the adverse effects of drought stress on plant growth by improving photosynthesis, activating the antioxidant defense system, and regulating the accumulation of osmotic substances.

Therefore, we hypothesize that the regulatory effects of exogenous selenium on tomato drought tolerance depend on the developmental stage and application dose. Moreover, selenium is expected to alleviate drought stress by enhancing photosynthesis, activating antioxidant defenses, and promoting osmotic adjustment.

2. Materials and Methods

2.1. Overview of the Experimental Site

The experiment was conducted from March to June 2024 in a solar greenhouse at the Xinxiang Comprehensive Experimental Base of the Chinese Academy of Agricultural Sciences (N 35°9', E 113°47', altitude 74 m). The experimental site has a warm temperate continental monsoon climate, with an average annual precipitation of 580 mm, annual evaporation of 2000 mm, average temperature of 14.1 °C, total sunshine duration of 2398.8 h, and a frost-free period of 201 d. The greenhouse used in this study covers a total area of 510 m² (60 m × 8.5 m), with an east-west orientation and a north-facing rear wall. It is covered with anti-drip polyethylene film, and the walls are embedded with 60 cm thick insulation material. A pot experiment was conducted using topsoil (0–20 cm) from open fields. The soil was sandy loam with a bulk density of 1.4 g·cm^{−3} and a field capacity of 23% (gravimetric water content). The soil contained 52.5 mg·kg^{−1} alkali-hydrolyzed nitrogen, 19.5 mg·kg^{−1} available phosphorus, and 196.1 mg·kg^{−1} available potassium. The soil pH was 8.6, electrical conductivity (EC) was 0.31 dS·m^{−1}, and organic matter content was 12.2 g·kg^{−1}.

2.2. Agronomic Management

Plastic pots used in the experiment were 30 cm in diameter and 50 cm in height. Air-dried soil was sieved through a 2 mm mesh and packed into the pots in three layers, with compaction after every 15 cm of soil. A base fertilizer was uniformly mixed into the upper soil layer, and pots were filled to a depth 3–5 cm below the rim. Each pot was filled with 42.5 kg of air-dried soil to ensure a bulk density of 1.4 g·cm^{−3}. Fertilizers were applied at rates of 0.13, 0.08, and 0.13 g·(kg dry soil)^{−1} for N, P₂O₅, and K₂O, respectively. The base fertilizer used was a conventional quick-release compound fertilizer (N:P₂O₅:K₂O = 19:19:19), applied at 30% of the total nitrogen requirement. All phosphorus fertilizer was applied as a basal dose, while the remaining 70% of nitrogen and the balance of potassium were split into three applications, fertigated at the onset of fruit enlargement for each fruit cluster. Fertilizer application rates were the same across all treatments. The tomato variety used for this study was Jingfan 404, a locally promoted variety widely cultivated in the study region. Seedlings at the 5-leaf stage were transplanted on 9 March 2024, with one plant per pot. Plants were pruned to retain three fruit clusters and topped after retaining three leaves above the last cluster. The experiment ended on 22 June 2024.

2.3. Experimental Design

The experiment was conducted following a two-factor completely randomized design (CRD), including four Se spraying periods and three irrigation control levels, resulting in

a total of 12 treatment combinations. The experimental design included four different Se spraying periods: T1 (spraying with water), T2 (Se spraying during the seedling stage), T3 (Se spraying during the flowering and fruit setting stages.), and T4 (Se spraying during both the seedling and flowering stages). For T2, Se was evenly sprayed twice on the plant leaves with a 7-day interval during the seedling stage. For T3, Se was evenly sprayed twice with a 20-day interval during the flowering and fruit setting stage. Each spraying continued until droplets formed on the leaf surface. The Se concentration ($2.5 \text{ mg}\cdot\text{L}^{-1}$) was determined based on previous research findings [13], using sodium selenite (Na_2SeO_3) as the selenium source. To prevent Na_2SeO_3 solution from dripping into the soil and affecting the experiment, waterproof plastic sheets were used to cover the soil surface during the exogenous Se spraying process. Each Se spraying treatment included three irrigation control levels, corresponding to 50–55%, 65–70%, and 80–85% of field capacity (designated as W1, W2, and W3, respectively), resulting in a total of 12 treatments when fully combined. To maintain different drought stress levels effectively, all treatments adopted a fixed irrigation quota system when the soil moisture content reached the lower limit of irrigation control. Specifically, the upper limits of irrigation were set at 100%, 85%, and 70% of field capacity, equating to an irrigation quota of 2.0 L for all treatments. For each treatment, 20 pots of uniform tomato seedlings were prepared to meet the requirements of destructive sampling and to minimize inter-plant variability, resulting in a total of 240 pots (12 treatments $\times$ 20 pots). Each pot contained one tomato plant and was considered an independent experimental unit. To ensure accurate sampling and reduce edge effects, the first two and last two pots in each treatment group were excluded, and three plants were randomly selected from the remaining pots as biological replicates for physiological and biochemical measurements. The mean values obtained from these three biological replicates were used for statistical analysis. To precisely control the irrigation volume for each treatment, an individual drip irrigation system was used, consisting of a watering bucket, a small self-priming pump, a pressure gauge, valves, capillaries, and drippers equipped with flow regulators (Figure 1). The emitter's rated flow rate was $2 \text{ L}\cdot\text{h}^{-1}$, operating at a pressure of 0.12 MPa. During irrigation, the required amount of water was added to the watering bucket, the self-priming pump was activated, and the working pressure was adjusted to 0.12 MPa to deliver water through the capillaries connected to the drippers into each pot until all the water in the bucket was used up. This meticulous setup ensured accurate control over Se application and irrigation, providing reliable data for analyzing the effects of Se spraying at different growth stages under various drought conditions on tomato plants.

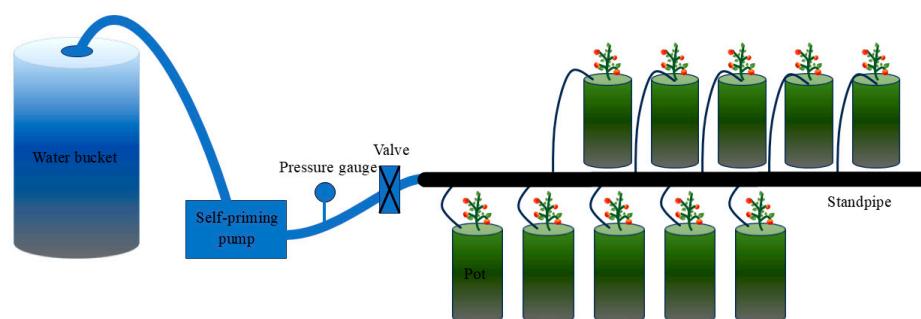


Figure 1. Irrigation System.

2.4. Main Observations and Methods

2.4.1. Plant Height, Stem Diameter, and Leaf Area

Fifteen days after Se foliar application during the flowering period, three representative tomato plants with similar overall growth from each treatment were selected

and marked for evaluating plant height, leaf area, and stem diameter. Plant height was measured using a ruler from the stem base to the growing point, while stem diameter was determined with calipers by measuring at 2 cm above the soil surface in perpendicular orientations. Leaf length and width were measured using a measuring tape, and leaf area (LA) was estimated using the product reduction coefficient method ($LA = \text{length} \times \text{width} \times 0.6393$) [22].

2.4.2. Aboveground Dry Matter

At the mature harvest stage, three plants of equal size were randomly selected from each treatment. These plants were segregated into stems, leaves, and fruits, and their fresh weights were measured separately. Subsequently, the samples were placed in an oven at 105 °C for 30 min to halt all biological activity and then dried to a constant weight at 75 °C. After drying, the dry weights of each part (stem, leaf, fruit) were measured individually. The water content of the fruits was calculated based on the difference between the fresh and dry weights.

2.4.3. Se Content

At fruit maturity and harvest, the aboveground parts (leaves and fruits) of plants from each treatment were collected. Samples were washed with distilled water, blotted dry, and combined with the remaining half of the fruit sample reserved for quality measurements. The samples were then placed in an oven, subjected to 30 min of heat treatment at 105 °C, and subsequently dried at 75 °C until constant weight was reached. The Se content in leaves and fruits was measured using inductively coupled plasma mass spectrometry (ICP-MS) [23]. For fruits, Se content was converted to a per unit fresh weight basis according to the fruit water content.

2.4.4. Photosynthesis and Related Physiological Parameters

Five days after Se foliar application during the flowering period, on a clear day between 9:00 and 11:00 a.m., the fourth fully expanded and well-grown functional leaf (from the top down) of each plant was selected. Photosynthetic rate (Pn), transpiration rate (Tr), and stomatal conductance (Gs) of tomato leaves under different treatments were measured using a Licor-6400 system (LI-COR Inc., Lincoln, NE, USA). Three representative plants per treatment were randomly selected for these measurements.

2.4.5. Photosynthetic Pigment Content

At 5 and 15 days after Se spraying during the flowering period, the well-grown functional leaf (from the top down) was selected from each plant. Chlorophyll was extracted in 95% ethanol under dark conditions for 24 h, and the absorbance of the extract was measured at 645 nm and 663 nm using a spectrophotometer [24]. In each set of measurements, three tomato plants with similar growth conditions were designated, and one marked leaf per plant was used. Three measurement points on each leaf were recorded and averaged. The specific formulas applied were as follows:

$$C_a = (12.7 \times A_{663} - 2.69 \times A_{645}) \times V/m \quad (1)$$

$$C_b = (22.7 \times A_{645} - 4.68 \times A_{663}) \times V/m \quad (2)$$

$$C_T = C_a + C_b \quad (3)$$

where C_a , C_b , and C_T represent the contents of chlorophyll a, chlorophyll b, and total chlorophyll ($\text{mg} \cdot \text{g}^{-1}$), respectively, A_{645} and A_{663} are the absorbance values of the chlorophyll

extract at 645 nm and 663 nm, V is the volume of the extraction solution (mL), and m is the mass of the leaf sample (mg)

2.4.6. Peroxidation Products and Osmoregulatory Substances

Five days after the application of exogenous Se, three healthy functional leaves from top to bottom of tomato plants were collected between 8:00 a.m. and 11:00 a.m. as three biological replicates for each treatment. The leaves were cleaned with deionized water to remove impurities, dried, then swiftly wrapped in aluminum foil and frozen in liquid nitrogen before being stored at -80°C . Biochemical analyses were subsequently conducted, including determining proline content using the acid ninhydrin method [25], measuring soluble sugar content by the anthrone colorimetric method [26], assessing superoxide dismutase (SOD) activity with the xanthine oxidase method [10], evaluating peroxidase (POD) activity through the guaiacol method [26], and quantifying glutathione peroxidase (GSH-Px) activity by a microdetermination method [10]. This comprehensive analysis aims to elucidate how Se application at different growth stages influences the antioxidant system and osmotic regulation in tomatoes, thereby providing insights into mechanisms that enhance drought tolerance and optimize crop yield under adverse environmental conditions [27].

2.5. Data Analysis

Data were collected and organized using Excel 2023. A two-way analysis of variance (ANOVA) was performed using SPSS 25.0 (IBM Corp., Armonk, NY, USA) to evaluate the main and interactive effects of selenium spraying period (T1–T4) and irrigation level (W1–W3) on physiological and biochemical parameters. Prior to ANOVA, data were tested for normality (Shapiro–Wilk test) and homogeneity of variances (Levene’s test) to ensure that ANOVA assumptions were met. Duncan’s new multiple range test was applied for post hoc multiple comparisons to identify significant differences among treatments at the 0.05 probability level. Graphs were generated using Origin 2022 (OriginLab Corp., Northampton, MA, USA).

3. Results

3.1. Growth Status of Tomato

As shown in Figure 2, T , W , and $T \times W$ significantly affected plant height and individual leaf area. W had a significant effect on stem diameter, whereas T and $T \times W$ showed no significant effects. Within the same Se treatment, plant height, stem diameter, and leaf area per plant all decreased with increasing drought severity. Under W3 conditions, compared with T1, T2 and T3 significantly increased plant height by 15.9% and 11.0%, respectively, while T4 showed no significant effect. Under the same conditions, T2, T3, and T4 significantly increased leaf area by 24.6%, 26.8%, and 9.8%, respectively, but none of the treatments had a significant effect on stem diameter. Under W2 conditions, plant height and leaf area in T2, T3, and T4 were higher than those in T1, with T2 showing the greatest improvement; however, no significant differences were observed among T2, T3, and T4. Under W1 conditions, compared with T1, T2, T3, and T4 significantly increased plant height by 9.5%, 13.7%, and 10.9%, respectively. Leaf area showed no significant changes under T2 and T4, but increased significantly by 32.5% under T3. These results indicate that Se spraying at any growth period promotes tomato growth, however, the growth promotion effect varies with the timing of Se application. Notably, dual-stage spraying (T4) was less effective than single-stage spraying, particularly under severe drought stress, where Se application during the flowering stage exhibited the most pronounced alleviation of drought effects.

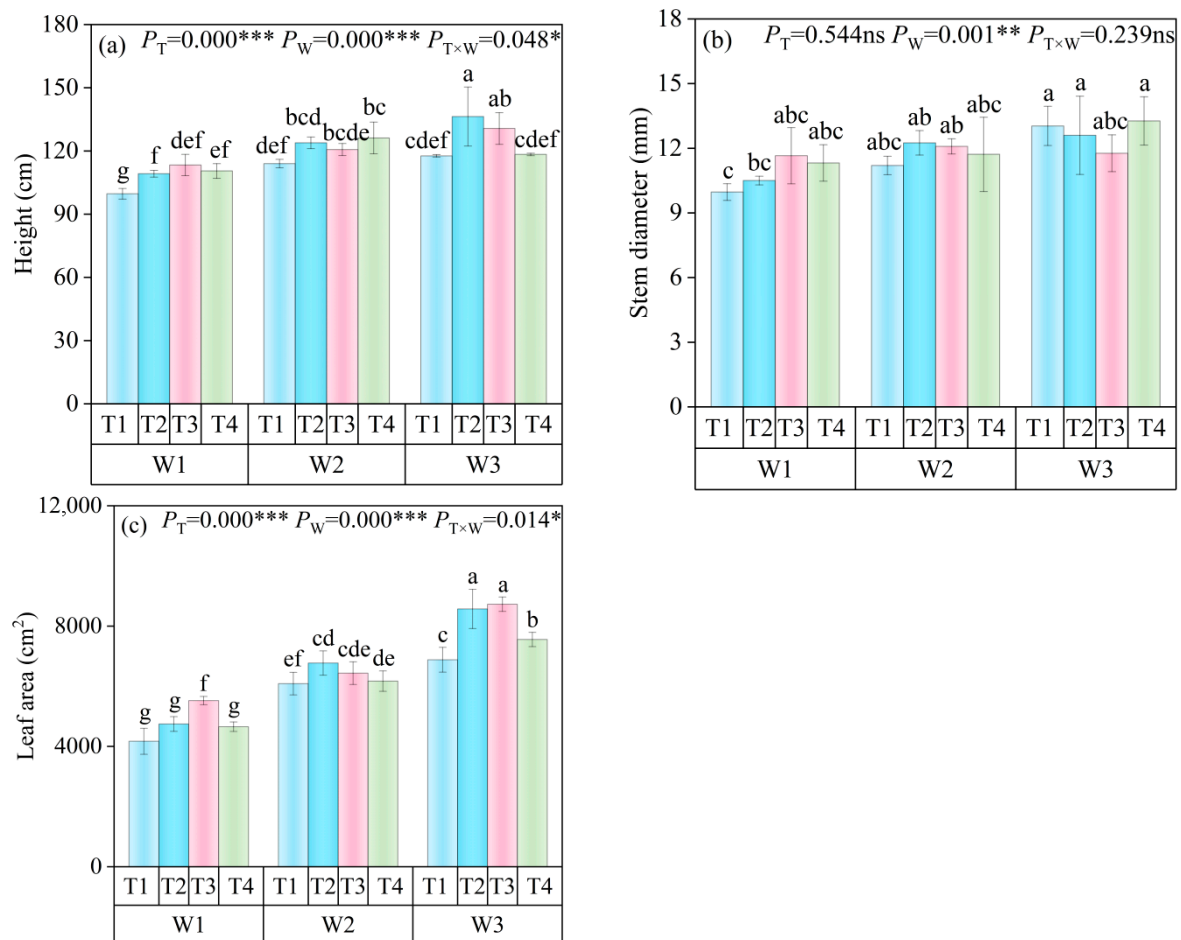


Figure 2. Changes in tomato plant height (a), stem diameter (b), and leaf area (c) following foliar selenium application. “ns”—non-significant ($p > 0.05$); “*”—significant at $p < 0.05$; “***”—extremely significant at $p < 0.01$; “****”—extremely significant at $p < 0.001$. The means with the same small case letters are statistically non-significant. P_T , P_W , and $P_{T \times W}$ represent the significance levels of the variance sources for the Se spraying period, irrigation level, and their interaction, respectively, according to two-way ANOVA.

3.1.1. Shoot Dry Biomass in Tomato

As shown in Figure 3, T and W significantly affected total aboveground dry matter, whereas $T \times W$ had no significant effect. Across different T and W treatments, the dry matter of fruits, leaves, and stems accounted for 44.1–52.1%, 19.9–23.6%, and 26.6–32.6% of the total aboveground biomass, respectively, with fruits representing the largest proportion. Within the same Se treatment, the dry matter of fruits, leaves, stems, and total aboveground biomass declined significantly with increasing drought severity. Under W3 and W2 conditions, Se spraying at all growth stages promoted dry matter accumulation, though the differences were not statistically significant. Under W1 conditions, compared with T1, total aboveground dry matter increased significantly by 54.8% and 38.2% under T3 and T4, respectively, while no significant difference was observed under T2. These results indicate that while under well-irrigated and mild drought conditions the stimulatory effect of Se spraying on dry matter accumulation is not pronounced, under severe drought stress all Se treatments significantly promoted the accumulation of aboveground dry biomass, with the most prominent effect observed during the flowering stage.

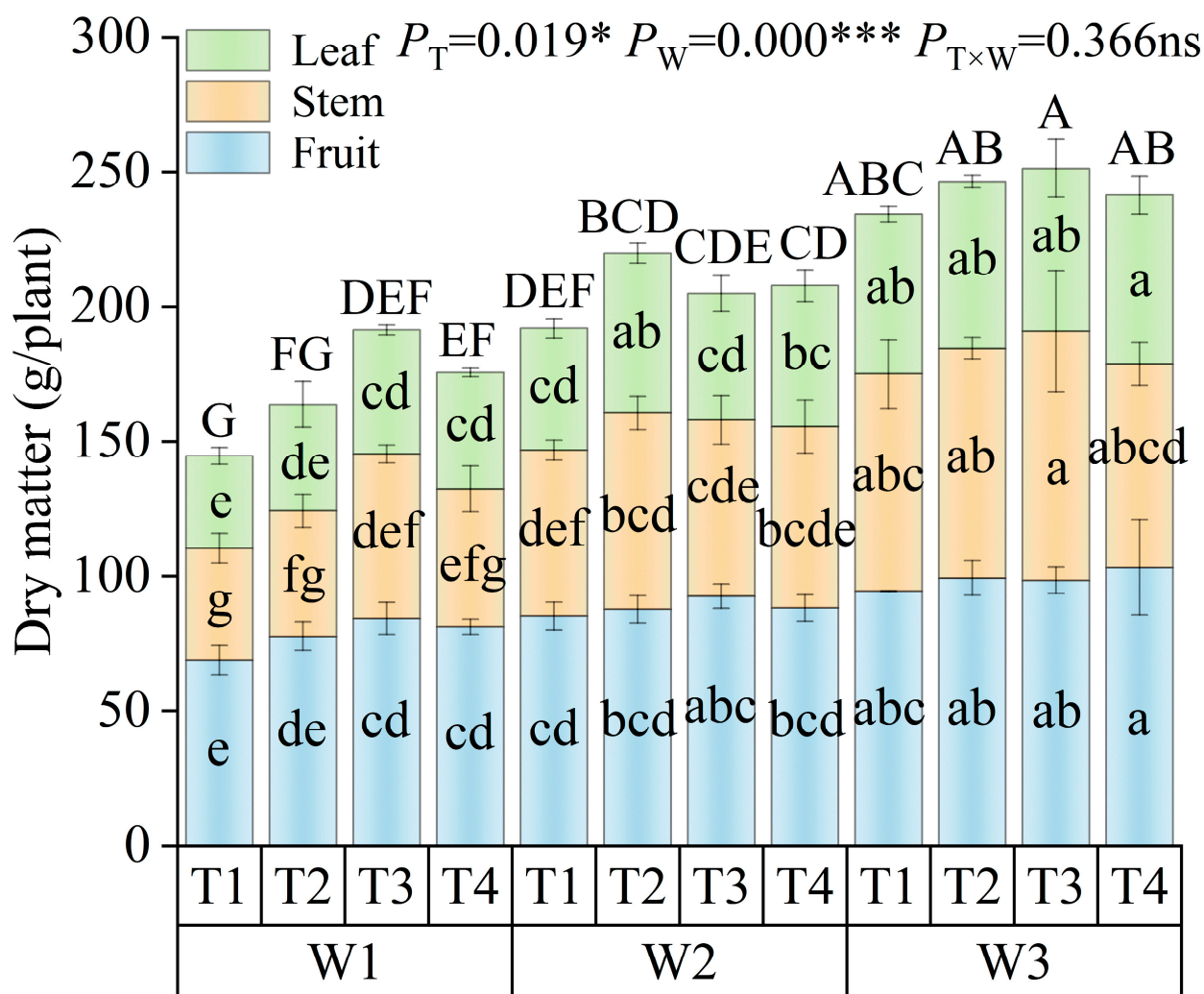


Figure 3. Dry matter of tomato organs following foliar selenium application. Capital letters above the first row of bars indicate statistical differences for total plant dry matter, while lowercase letters in the subsequent three rows indicate statistical differences for individual organs (leaf, stem, and fruit, respectively). “ns”—non significant ($p > 0.05$); “*”—significant at $p < 0.05$; “***”—extremely significant at $p < 0.001$. The means with the same small case letters are statistically non-significant. P_T , P_W , and $P_{T \times W}$ represent the significance levels of the variance sources for Se spraying period, irrigation level, and their interaction, respectively, according to two-way ANOVA.

3.1.2. Se Content in Different Tomato Organs

As shown in Figure 4, Se content in tomato leaves and fruits was extremely significantly affected by T, W, and $T \times W$. Under the same water conditions, Se application at different growth stages resulted in significant differences in Se accumulation and translocation in tomato leaves and fruits. In addition, under identical water regimes, the timing of Se application significantly influenced both Se accumulation and conversion efficiency in tomatoes. The results indicate that foliar Se application during the seedling stage (T2) notably enhanced Se uptake less than that during the flowering stage (T3) or the dual-stage treatment (T4), suggesting that the flowering and fruit-setting stage may be the critical period for Se absorption and translocation in tomato plants.

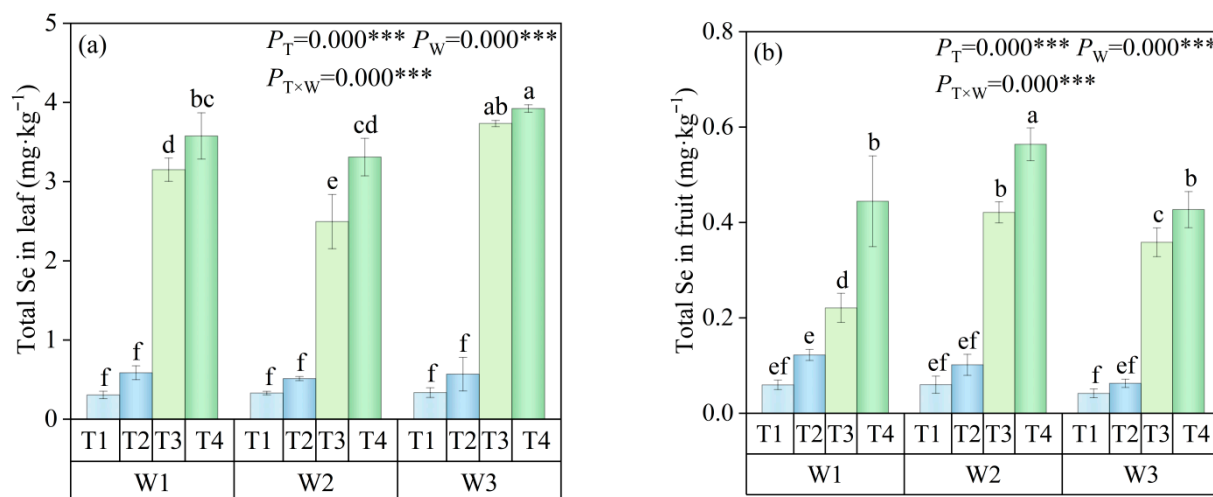


Figure 4. Selenium content in tomato leaves (a) and fruits (b) following foliar selenium application. “***” extremely significant at $p < 0.001$. The means with the same small case letters are statistically non-significant. P_T , P_W , and $P_{T \times W}$ represent the significance levels of the variance sources for Se spraying period, irrigation level, and their interaction, respectively, according to two-way ANOVA.

3.2. Effects of Se on Tomato Photosynthetic Characteristics

3.2.1. Leaf Photosynthesis

As shown in Table 1, both T and W had highly significant effects on net photosynthetic rate (Pn), stomatal conductance (Gs), and transpiration rate (Tr), while $T \times W$ significantly affected Gs and Tr. Under the same Se application timing, tomato leaf Pn, Gs, and Tr decreased with increasing drought stress. Under the T1 condition, compared with the well-irrigated (W3) condition, the W2 treatment significantly reduced Pn, Gs, and Tr by 6.3%, 30.3%, and 11.0%, respectively, while the W1 treatment significantly reduced these parameters by 14.9%, 18.4%, and 24.0%, respectively. Under sufficient irrigation (W3), T4 resulted in the highest Pn, although no significant differences were observed among the Se treatments. Under mild drought stress (W2), all Se treatments significantly improved the photosynthetic characteristics of tomato plants, with T3 showing the most pronounced enhancement in Gs (an increase of 36.0% compared with the control), while T4 exhibited the highest Pn. Under severe drought stress (W1), compared with T1, Pn in T2, T3, and T4 increased by 2.9%, 5.6%, and 6.3%, respectively, and Tr increased by 23.7%, 39.1%, and 25.9%, respectively. Although no significant difference in Pn was observed between T3 and T4 under severe drought, T3 achieved the highest Gs and Tr. These results indicate that under sufficient irrigation Se application did not significantly enhance tomato photosynthesis, however, under severe drought stress, Se application during the flowering stage (T3) markedly improved photosynthetic efficiency.

Table 1. Net photosynthetic rate (Pn), stomatal conductance (Gs), and transpiration rate (Tr) in tomato leaves following foliar selenium application.

Treatment		Pn (μmolCO ₂ ·m ⁻² ·s ⁻¹)	Gs (molH ₂ O·m ⁻² ·s ⁻¹)	Tr (molH ₂ O·m ⁻² ·s ⁻¹)
W1	T1	36.17 ± 0.74 g	0.56 ± 0.09 cdef	7.13 ± 0.10 g
	T2	37.20 ± 0.38 f	0.47 ± 0.04 f	8.82 ± 0.42 ef
	T3	38.21 ± 0.86 e	0.58 ± 0.02 bcde	9.92 ± 0.60 cd
	T4	38.47 ± 0.49 e	0.50 ± 0.07 def	8.97 ± 0.63 ef

Table 1. Cont.

Treatment		Pn ($\mu\text{molCO}_2 \cdot \text{m}^{-2} \cdot \text{s}^{-1}$)	Gs ($\text{molH}_2\text{O} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$)	Tr ($\text{molH}_2\text{O} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$)
W2	T1	39.81 $\pm$ 0.14 d	0.48 $\pm$ 0.07 ef	8.35 $\pm$ 0.38 f
	T2	40.45 $\pm$ 0.54 d	0.63 $\pm$ 0.04 bc	9.58 $\pm$ 0.37 de
	T3	40.72 $\pm$ 0.45 d	0.65 $\pm$ 0.09 abc	10.19 $\pm$ 0.62 bcd
	T4	42.11 $\pm$ 0.22 c	0.60 $\pm$ 0.04 bcd	10.86 $\pm$ 0.30 ab
W3	T1	42.49 $\pm$ 0.37 bc	0.69 $\pm$ 0.06 ab	9.38 $\pm$ 0.43 de
	T2	42.80 $\pm$ 0.89 abc	0.63 $\pm$ 0.05 bc	10.91 $\pm$ 0.34 ab
	T3	43.37 $\pm$ 0.14 ab	0.75 $\pm$ 0.05 a	11.64 $\pm$ 0.52 a
	T4	43.68 $\pm$ 0.66 a	0.61 $\pm$ 0.02 bcd	10.47 $\pm$ 0.49 bc
p	T	0.000 ***	0.005 **	0.000 ***
	W	0.000 ***	0.000 ***	0.000 ***
	T $\times$ W	0.266 ns	0.022 *	0.021 *

Note: “ns”—non-significant ($p > 0.05$); “*”—significant at $p < 0.05$; “**”—extremely significant at $p < 0.01$; “***”—extremely significant at $p < 0.001$. P_T , P_W , and $P_{T \times W}$ represent the significance levels of the variance sources for Se spraying period, irrigation level, and their interaction, respectively, according to two-way ANOVA. Data are presented as means $\pm$ SE ($n = 3$). Within each column, means sharing the same lowercase letter are not significantly different.

3.2.2. Chlorophyll Changes

As shown in Figure 5, both T and W exerted highly significant effects on leaf chlorophyll content (total chlorophyll unless otherwise specified). T $\times$ W showed a significant effect at 5 days after Se spraying, but this effect was not observed at 15 days. Under W3 conditions, no significant differences in chlorophyll content were detected among the Se treatments compared with T1 at either 5 or 15 days after spraying. Under W2 conditions, chlorophyll b in T3 did not differ significantly from T1 at 5 days, while chlorophyll a reached its maximum, indicating a preferential enhancement of chlorophyll a synthesis. chlorophyll content increased markedly under T3 but decreased under T4 at 5 days; by day 15, no significant differences were found among T2, T3, T4, and T1. Under W1 conditions, chlorophyll content in T3 and T4 remained significantly higher than T1 at both 5 and 15 days. Although T2 showed no difference from T1 at 5 days, chlorophyll a content increased significantly by day 15, suggesting stimulation of chlorophyll a synthesis. Overall, under well-irrigated conditions, Se spraying has a limited effect on enhancing chlorophyll synthesis in tomato leaves. Under both severe and mild drought stress, Se application significantly promotes chlorophyll synthesis, exhibiting clear time-dependent characteristics: in the short term, Se application during the flowering stage (T3) was most effective, whereas in the long term the effects of the various Se treatments tended to converge.

3.3. Antioxidant Capacity and Osmoregulatory Substances in Tomato Leaves

3.3.1. Activities of Superoxide Dismutase (SOD), Peroxidase (POD), and Glutathione Peroxidase (GSH-PX) in Leaves

As shown in Figure 6, T and T $\times$ W exerted significant effects on leaf SOD, POD, and GSH-Px activities. W significantly affected SOD and GSH-Px activities, but its influence on POD was not significant. Under the non-Se treatment (T1), leaf SOD and GSH-Px activities increased significantly with the severity of drought stress (Figure 6a,c). In contrast, leaf POD activity initially increased and then decreased as drought stress intensified (Figure 6b). Under W3 and W2 conditions, the T3 treatment produced the highest increases in leaf SOD activity. In these conditions, all Se treatments significantly reduced leaf POD activity while significantly increasing leaf GSH-Px activity, indicating an opposing trend in the effects on POD and GSH-Px activities. W1 conditions, compared with T1, T2 and T3 treatments, enhanced leaf SOD activity by 12.4% and 57.7%, respectively, with the enhancement in

T3 being 4.7 times that in T2. Meanwhile, under T2, T3, and T4 treatments, leaf POD activity increased by 33.2%, 12.9%, and 12.8% compared with T1, while GSH-Px activity increased by 5.5%, 2.3%, and 31.2%, respectively. These results indicate that following complete Se spraying, the regulatory effects of Se application at different growth stages on key antioxidant enzymes differ. Under well-irrigated and mild drought conditions, GSH-Px appears to play a dominant role in scavenging H_2O_2 , with its activity increasing in line with the “amount of Se sprayed.” Under severe drought stress, however, Se application during the seedling stage primarily induces an upregulation of SOD activity, followed by a coordinated enhancement of both POD and GSH-Px activities to alleviate drought stress.

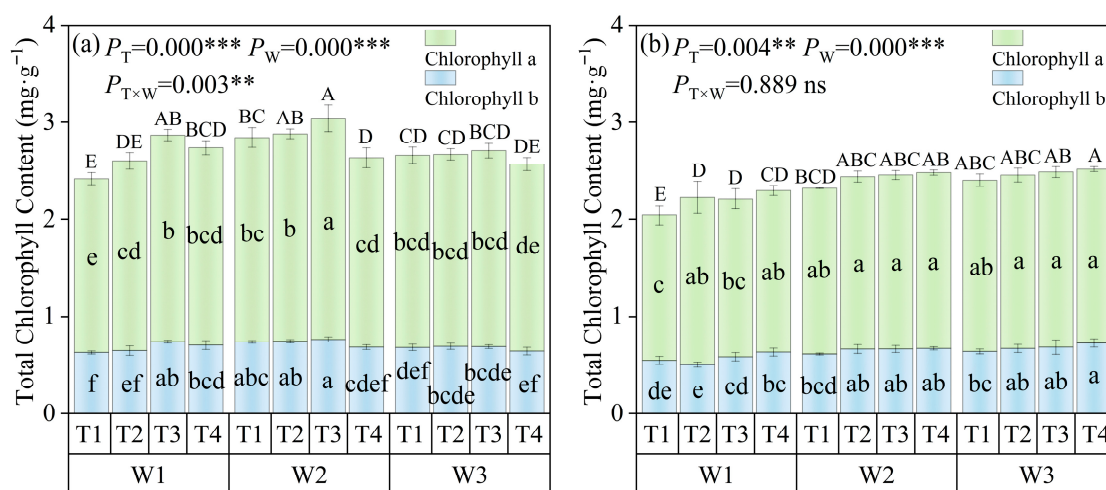


Figure 5. Dynamic changes in total chlorophyll content in tomato leaves at 5 days (a) and 15 days (b) following foliar selenium application. Capital letters above the first row of bars indicate statistical differences for total chlorophyll content, while lowercase letters in the subsequent two rows indicate statistical differences for chlorophyll a and chlorophyll b, respectively. “ns”—non significant ($p > 0.05$); “***”—extremely significant at $p < 0.01$; “****”—extremely significant at $p < 0.001$. The means with the same small case letters are statistically non-significant. P_T , P_W , and $P_{T \times W}$ represent the significance levels of the variance sources for Se spraying period, irrigation level, and their interaction, respectively, according to two-way ANOVA.

3.3.2. Effects of Exogenous Se on Osmoregulatory Substances in Tomato Leaves

As shown in Figure 7, T, W, and $T \times W$ significantly influenced leaf proline and soluble sugar contents. Under the non-Se treatment (T1), both proline and soluble sugar contents increased significantly with increasing drought stress (Figure 7a,b). Compared with the well-irrigated condition (W3), the W2 treatment resulted in significant increases in proline and soluble sugar contents by 448.7% and 51.0%, respectively, whereas the W1 treatment led to significant increases of 1140.2% in proline and 16.0% in soluble sugars. Under W3 and W2 conditions, T2, T3, and T4 all exhibited significantly higher proline and soluble sugar contents compared with T1, although proline content did not differ significantly among T2, T3, and T4. Under W1 conditions, compared with T1, T2 and T3 increased proline content by 14.2% and 16.5%, respectively, whereas soluble sugar content decreased by 15.8% and 13.4%, respectively. T4 showed no significant change in proline content but significantly increased soluble sugar content. These results indicate that the regulatory effects of Se spraying on the osmoregulatory system vary with the timing of application. The experimental data also demonstrate that proline content is highly sensitive to soil water status, and an appropriate level of Se spraying can significantly enhance crop osmoregulatory capacity through increased proline accumulation.

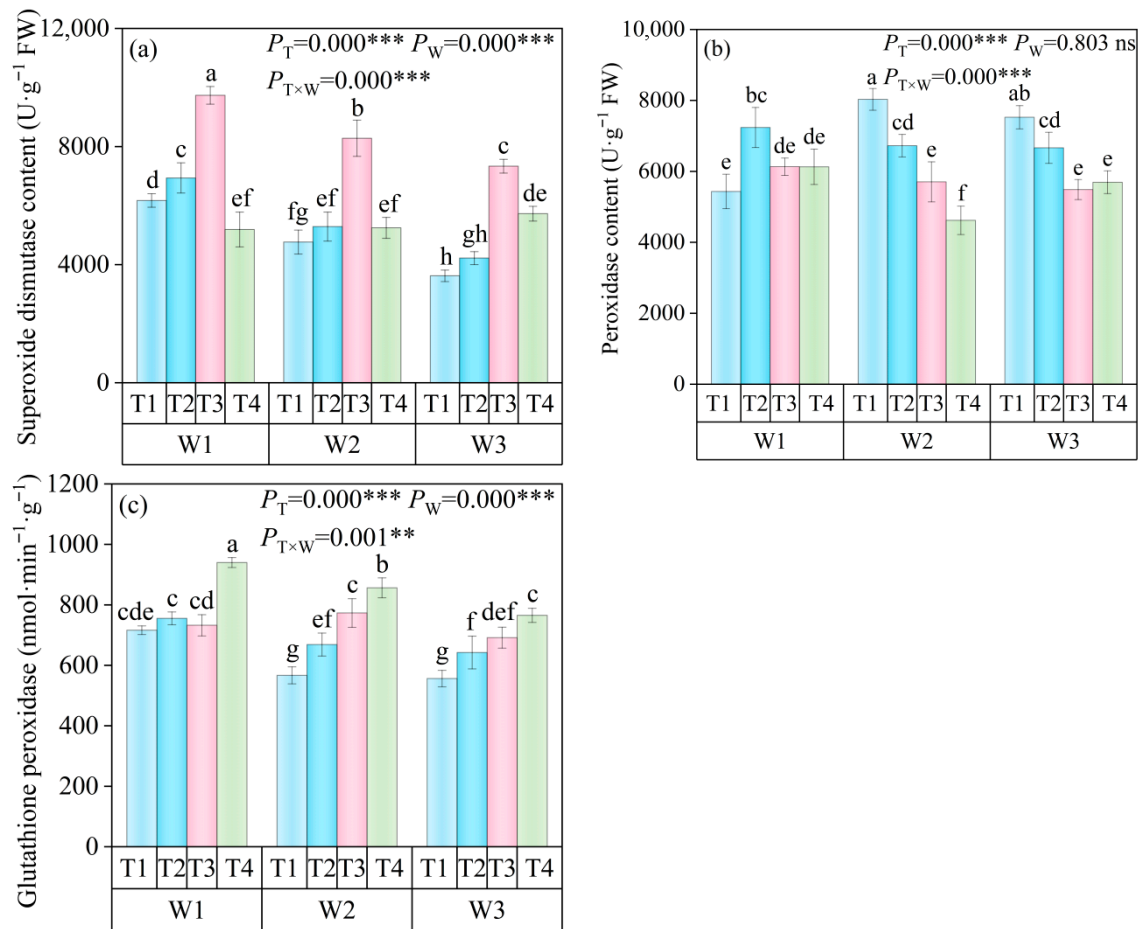


Figure 6. Tomato leaf SOD (a), POD (b), and GSH-Px (c) activity following foliar selenium application. “ns”—non-significant ($p > 0.05$); “***”—extremely significant at $p < 0.01$; “****”—extremely significant at $p < 0.001$. The means with the same small case letters are statistically non-significant. P_T , P_W , and $P_{T \times W}$ represent the significance levels of the variance sources for Se spraying period, irrigation level, and their interaction, respectively, according to two-way ANOVA.

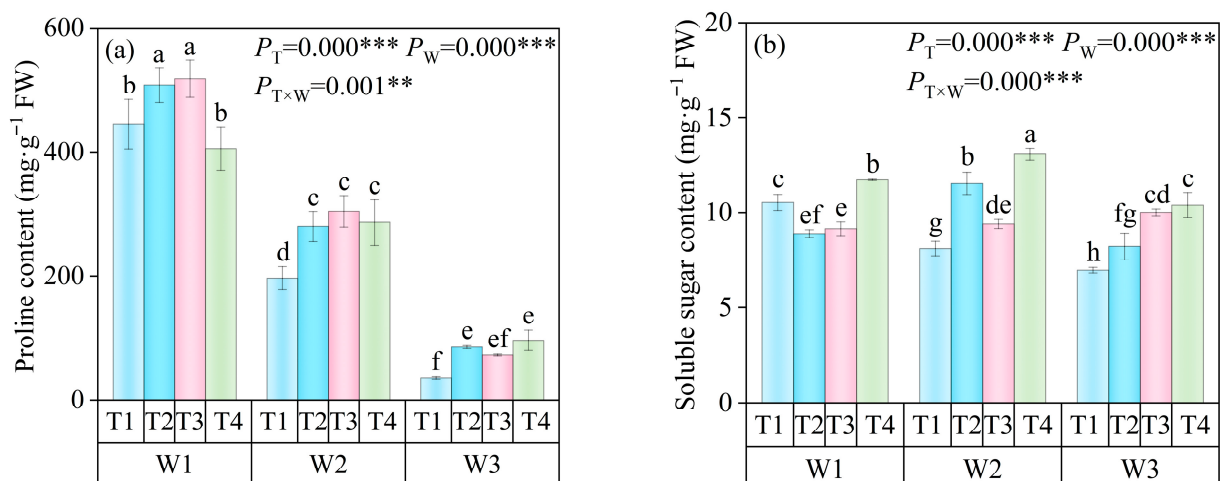


Figure 7. Proline (a) and soluble sugar (b) contents in tomato leaves following foliar selenium application. “***”—extremely significant at $p < 0.01$; “****”—extremely significant at $p < 0.001$. The means with the same small case letters are statistically non-significant. P_T , P_W , and $P_{T \times W}$ represent the significance levels of the variance sources for Se spraying period, irrigation level, and their interaction, respectively, according to two-way ANOVA.

4. Discussion

Plants can effectively mitigate oxidative damage from reactive oxygen species (ROS) through the coordinated regulation of antioxidant defense systems and osmoregulatory mechanisms [28]. The antioxidant defense system comprises both enzymatic components (e.g., key enzymes such as SOD, POD, CAT, APX, and GR) and non-enzymatic components (e.g., antioxidants such as AsA and GSH) [29]. In this study, it was observed that, with increasing drought stress, the activities of SOD and GSH-Px in leaves of the non-Se treatment (T1) were significantly elevated (Figure 6a,c), which is consistent with the findings of Hussain [30]. However, POD activity exhibited an initial increase followed by a decrease, which is inconsistent with the conclusions of Sun [31]. This discrepancy might be attributed to a more prominent role of GSH-Px in H_2O_2 scavenging under these conditions [32]. Furthermore, as a key osmoregulatory substance in the response to abiotic stress, the changes in proline content directly reflect the dynamic balance between stress intensity and plant drought tolerance [33]. In our study, the contents of proline and soluble sugars in leaves of the non-Se treatment (T1) increased significantly with the intensification of drought stress (Figure 7a,b), a phenomenon that aligns with the conclusions of Khan et al. [34]. The accumulation of these osmoregulatory substances helps to maintain the stability of the cell membrane system and mitigate damage caused by drought stress.

Se plays an important role in alleviating the excessive production of ROS induced by drought stress by stimulating both enzymatic and non-enzymatic antioxidants to alleviate oxidative stress and promote photosynthetic performance [35]. Studies by Hartikainen and Hussain further confirm that exogenous Se can significantly enhance the activities of antioxidant enzymes (e.g., POD and SOD), thereby promoting ROS scavenging and mitigating the harmful impacts of drought and other abiotic stresses [16]. Our results indicate that the regulatory effects of Se application on both the antioxidant system and osmoregulatory system under varying degrees of drought stress are not entirely consistent. This discrepancy may be due to differences in the ROS levels in the plant tissues and the induced antioxidant enzyme activities under different drought intensities [36]. Under severe drought stress, Se spraying during the flowering and fruit-setting stage significantly increased SOD activity, suggesting that under such conditions the plant predominantly enhances drought tolerance via the regulation of SOD. In contrast, under the seedling stage treatment, POD activity reached its highest value, which may be attributed to the early induction and upregulation of SOD during the seedling stage. As the first line of defense against oxidative stress, SOD quickly initiates the dismutation of superoxide anion radicals, resulting in the production of H_2O_2 that subsequently activates POD to accelerate the repair of oxidative damage [37,38]. Additionally, under dual-stage Se spraying, the content of GSH-Px was significantly increased (Figure 6c). This indicates that GSH-Px plays a dominant role in H_2O_2 scavenging under these conditions, thereby potentially reducing the demand for SOD and POD activities [29]. Conversely, during the seedling stage Se spraying treatment, if the Se content is insufficient, it can lead to inadequate GSH-Px activity. In this case, POD is further activated to catalyze the degradation of H_2O_2 , preventing its excessive accumulation within cells which could otherwise result in oxidative damage [39]. As an integral component of various enzymes and proteins, Se is involved in regulating the activity of glutathione peroxidase (GSH-Px) and, together with ascorbic acid (AsA) acting as a reducing agent, reduces hydrogen peroxide and other peroxides—thereby exhibiting a strong antioxidant capacity [40,41]. With increasing soil moisture, under mild drought and well-irrigated conditions, the enhancement effects of different Se application timings on GSH-Px activity in tomato leaves varied, while the changes in POD activity were negatively correlated with those in GSH-Px activity. This suggests that under such conditions GSH-Px plays a dominant role in H_2O_2 removal, with any residual H_2O_2 being

further degraded by POD to prevent the accumulation of oxidative damage, which is consistent with the findings of Feng [33]. In summary, regardless of the growth stage at which Se is applied, foliar spraying can rapidly adjust the antioxidant system, increase the activities of antioxidant enzymes, and thereby alleviate drought-induced damage to the membrane system.

Under drought stress, plants typically increase proline synthesis to function as an osmoregulator, thereby helping to maintain intracellular water balance [16]. This study showed that the proline content in tomato leaves gradually increased with increasing drought stress (Figure 7a), reaching 6.5 times that under well-irrigated conditions (W3) when under severe drought (W1). This finding is consistent with the conclusions of Yaish et al. regarding the regulation of cell turgor by proline [42]. Existing studies indicate that stress-induced proline accumulation can enhance plant tolerance by maintaining osmotic balance, regulating redox homeostasis, supplying energy, and mediating signal transduction [43–46]. Khan et al. [47] demonstrated that the combined application of Se and 24-epibrassinolide (EBL) could elevate proline levels in wheat under combined heat and drought stress, thereby improving growth and photosynthetic performance. These synergistic effects suggest that Se may serve as a signaling cofactor to enhance osmolyte accumulation and stress-responsive pathways [48]. In our study, although all Se spraying treatments (at various growth stages) increased proline and soluble sugar contents under well-irrigated conditions (Figure 7a,b), these increases did not significantly affect photosynthetic performance or dry matter accumulation—suggesting that accumulation of osmoregulatory substances may serve primarily a prophylactic protective role under such conditions. This preventive accumulation may prime plants for potential future stress events, a phenomenon consistent with the concept of “stress memory” or pre-conditioning reported in Se-treated crops. Under severe drought stress, however, treatments W1T2 and W1T3 significantly enhanced proline content compared with W1T1, and the aboveground dry matter accumulation was notably increased under T2 and T3. These results indicate that Se can enhance plant drought tolerance under drought stress. The observed decrease in soluble sugar content might be related to the mechanism reported by Khan et al. [49], in which Se upregulates the activity of proline synthase (glutamine kinase), with soluble sugars being degraded to provide ATP and NADPH that promote proline synthesis [50]. Moreover, the dual-stage spraying treatment (T4) under severe drought did not improve proline levels as effectively as the single-stage treatments (T2/T3), indicating that an excessive “spraying dose” or frequent spraying does not continuously benefit the plant’s defense against oxidative damage. In summary, considering the growth stage characteristics of tomato, a single Se treatment during the seedling stage or the flowering and fruit-setting stage is an effective strategy to enhance drought tolerance by optimizing osmotic regulation.

It has been shown that drought stress significantly reduces leaf relative water content, leading to chlorophyll degradation and growth inhibition [51]. Our long-term drought treatments (Table 1) confirmed that net photosynthetic rate (Pn), stomatal conductance (Gs), transpiration rate (Tr), and total chlorophyll content in tomato leaves all decreased with increased drought stress. Chlorophyll, a key photosynthetic pigment, shares the glutamate metabolic pathway with proline synthesis [52,53]. In our study, as drought stress intensified, the proline content in tomato leaves increased significantly (Figure 7a), while chlorophyll content decreased markedly (Figure 5a,b), the two exhibited a negative correlation under stress conditions. This finding is consistent with the results reported by Yaish [42] and Osakabe [54]. Although the regulatory mechanisms underlying their stress responses remain to be fully elucidated, chlorophyll content, as an important index of the plant’s photosynthetic capacity [55], together with proline levels reflecting stress intensity, constitute a crucial indicator system for evaluating plant drought tolerance [45,56]. This

study also revealed that the effects of Se spraying are significantly specific to different growth stages. Under well-irrigated conditions, Se spraying at different periods showed no significant impact on chlorophyll synthesis, photosynthetic parameters, or dry matter accumulation. However, under severe drought conditions, Se spraying at any period resulted in increased chlorophyll content, photosynthetic parameters, and dry matter accumulation in tomato leaves compared to no Se spraying. Particularly, Se spraying during the flowering stage exhibited the most significant promotion effect. These results are consistent with previous studies [57]. Further analysis indicated that the increase in Tr following Se treatment was positively correlated with the accumulation of aboveground biomass. This result is consistent with the findings of Zhang and Tao [58,59], who reported that Se treatment can indirectly promote water evaporation from leaf surfaces by enhancing photosynthetic efficiency. These findings contrast with studies reporting reduced stomatal numbers and transpiration under drought stress, suggesting that Se may enhance drought tolerance via unique physiological mechanisms [60].

Drought stress can significantly affect plant physiological activities, leading to slower growth, reduced photosynthetic efficiency, and metabolic disorders [13]. Our results demonstrated that drought stress significantly reduced tomato plant height, stem diameter, individual leaf area, and aboveground dry matter accumulation, which is in agreement with the findings of Zhong [13]. Under osmotic stress, stomatal closure hinders CO₂ assimilation, thereby reducing the demand for NADPH in the Calvin cycle [61]. At this time, the chloroplastic P5CS recycles NADP⁺ to synthesize proline precursors, thereby sustaining the operation of the electron transport chain while reducing ROS production in photosystem I [62]. This study found that under well-irrigated conditions, Se spraying at various periods had limited effects on promoting dry matter accumulation. However, under severe drought stress, the above-ground dry matter of the W1T3 treatment was significantly higher than that of the W1T1 treatment. Additionally, there was no significant difference between the W1T3 and W2T1 treatments. Moreover, 5 days after the completion of Se spraying, the chlorophyll content in the W1T3 treatment showed a significant increase, while proline content decreased by 163.3%. This indicates that Se spraying during the flowering and fruit setting stage can effectively alleviate oxidative damage, possibly due to the plant's sensitivity to redox balance during this period. These findings are consistent with those of Neysanian et al. [36]. These changes suggest that foliar Se application during the flowering and fruit-setting period can, by influencing plant physiological and biochemical processes, induce greater drought tolerance and thereby better help plants cope with drought stress.

Additionally, this study found that foliar application of Se significantly increased the Se content in the above-ground organs of tomato plants, with the most significant effects observed during the flowering and fruit setting stage and dual-stage spraying (seedling and flowering stages) (Figure 4). Similar results have been observed in other crops. For instance, a study by De Vita et al. demonstrated that compared to the jointing stage, foliar Se application during the heading stage significantly increased the average Se concentration in wheat grains [63]. However, Nawaz et al. found that under drought stress conditions, Se application during the tillering stage resulted in a more significant increase in wheat yield compared to Se application during the flowering stage [64], a difference that may be related to the growth characteristics of the crop's fruit. Meucci et al. [21] further noted in their study that the efficiency of Se uptake by tomatoes varies significantly across different growth stages. According to their findings, the flowering and fruit setting stage is the optimal period for Se application aimed at nutritional enhancement. Spraying Se during this period maximizes Se accumulation in the fruits. This timing not only ensures the

highest Se enrichment in the harvestable parts of the plant but also avoids potential toxicity risks that could arise if Se were applied during the vegetative growth stage.

In summary, this study provides new insights into the optimal timing for Se application in tomato during the seedling and flowering/fruit-setting stages. It reveals the mechanisms by which Se alleviates drought stress through improvements in photosynthesis, activation of the antioxidant defense system, and regulation of osmoregulatory substance accumulation. By comprehensively considering the complex interactions among Se supplementation, growth stage, and irrigation conditions, our research not only deepens the understanding of Se-mediated stress alleviation mechanisms but also provides a scientific basis for practical applications. Furthermore, these outcomes contribute to bridging the knowledge gap between physiological responses and agronomic management, offering valuable guidance for integrating Se foliar spraying into drought adaptation strategies. These findings can help optimize agricultural practices to enhance crop productivity and adaptability. Nevertheless, this study has limitations—for example, only a single Se concentration was evaluated, which does not fully reflect the effects of different concentrations on various growth stages. Given the differences in growth status between the tomato seedling and flowering stages, future studies should incorporate dynamic monitoring techniques to further explore the optimal timing and frequency of Se application, thereby enhancing the precision and efficacy of Se use. In addition, total chlorophyll (Chl a + b) was used as a general indicator of leaf pigment content and photosynthetic potential. Future work could further investigate the deeper effects of Chl a and b on light-harvesting complexes under Se application. This will provide a reference for developing more scientifically sound Se fertilizer management strategies to more effectively promote crop growth and stress tolerance.

5. Conclusions

This study systematically evaluated the effects of foliar application of sodium selenite at different growth stages on tomato drought tolerance, revealing clear growth-stage specificity. Application during the flowering and fruit-setting stage produced the most favorable outcomes, enhancing antioxidant enzyme activities, osmolyte accumulation, photosynthetic performance, and dry matter production. Seedling-stage application activated the antioxidant system but resulted in limited Se accumulation, while dual-stage application caused excessive Se and suppressed growth. Application at the flowering and fruit-setting stage also maximized Se enrichment in fruits while avoiding potential toxicity associated with vegetative-stage application, highlighting the optimal timing for improving both stress tolerance and nutritional quality.

Future research should focus on: (1) optimizing Se dose and application strategies; (2) exploring synergistic interactions with other micronutrients or regulatory factors; and (3) evaluating Se application across diverse environmental conditions. These findings provide guidance for precise Se fertilizer management and advance understanding of Se's role in enhancing plant stress tolerance and nutritional quality, contributing to efficient and sustainable horticultural production.

Author Contributions: Conceptualization, F.G.; methodology and software, H.L. (Huanhuan Li) and X.Q.; formal analysis, H.C.; investigation, H.C. and Y.Z.; data curation, H.C.; writing—original draft preparation, H.C.; writing—review and editing, H.L. (Hao Liu) and F.G.; writing guidance, G.W. and L.S.; funding acquisition, G.W. and H.L. (Hao Liu). All authors have read and agreed to the published version of the manuscript.

Funding: This research was supported by the National Natural Science Foundation of China (No. 52279052), the Natural Science Foundation of Shandong Province (No. ZR202102220203), the Natural Science Foundation of Henan Province (No. 252300421577), the Henan Province Scientific and Technological Research Project (No. 242102110187), and the Agricultural Science and Technology Innovation Program.

Data Availability Statement: The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation. The data are not publicly available due to copyright restrictions.

Conflicts of Interest: The authors declare no conflicts of interest.

References

1. Kizito, J. Evaluating the Influence of Two Organic Manures on the Productivity of Five Tomato Varieties. *OALib* **2024**, *11*, e12632. [\[CrossRef\]](#)
2. Dawid, J. The Role of Tomato Products for Human Health (*Solanum lycopersicum*)—A Review. *J. Health Med. Nurs.* **2016**, *33*, 66–74.
3. An, C.-H.; Ri, H.-J.; Han, T.-U.; Kim, S.-I.; Ju, U.-S. Feasibility of Winter Cultivation of Fruit Vegetables in a Solar Greenhouse in Temperate Zone; Experimental and Numerical Study. *Sol. Energy* **2022**, *233*, 18–30. [\[CrossRef\]](#)
4. Li, H.; Song, J.; Sun, J.; Wang, J.; Qiang, X.; Liu, H.; Zheng, M.; Lou, Y. Effects of Different Water and Nitrogen Supply on Fruit Nutrients and Yield Components of Greenhouse Tomato at Different Truss Levels. *J. Irrig. Drain.* **2023**, *42*, 1–9. (In Chinese) [\[CrossRef\]](#)
5. Zhang, P.; Zhang, Z.; Xiao, M.; Chao, J.; Dai, Y.; Liu, G.; Senge, M. Effects of Organic Mulching on Soil Moisture and Temperature in Greenhouse Tomato Production under Unheated Greenhouse Cultivation in the Cold Zone of China. *Food Sci. Nutr.* **2023**, *11*, 4829–4842. [\[CrossRef\]](#)
6. Fahad, S.; Bajwa, A.A.; Nazir, U.; Anjum, S.A.; Farooq, A.; Zohaib, A.; Sadia, S.; Nasim, W.; Adkins, S.; Saud, S.; et al. Crop Production Under Drought and Heat Stress: Plant Responses and Management Options. *Front. Plant Sci.* **2017**, *8*, 1147. [\[CrossRef\]](#)
7. Hussain, H.A.; Hussain, S.; Khaliq, A.; Ashraf, U.; Anjum, S.A.; Men, S.; Wang, L. Chilling and Drought Stresses in Crop Plants: Implications, Cross Talk, and Potential Management Opportunities. *Front. Plant Sci.* **2018**, *9*, 393. [\[CrossRef\]](#)
8. Song, J.; Li, H.; Xin, L.; Shen, J.; Liu, H.; Wang, X.; Zhong, Y. Effects of Foliar Selenium Application and Soil Moisture Interaction on Growth and Water Use Efficiency of Greenhouse Tomato. *J. Irrig. Drain.* **2023**, *42*, 52–59. (In Chinese) [\[CrossRef\]](#)
9. Chołuj, D.; Karwowska, R.; Ciszewska, A.; Jasińska, M. Influence of Long-Term Drought Stress on Osmolyte Accumulation in Sugar Beet (*Beta vulgaris* L.) Plants. *Acta Physiol. Plant* **2008**, *30*, 679–687. [\[CrossRef\]](#)
10. Zahedi, S.M.; Moharrami, F.; Sarikhani, S.; Padervand, M. Selenium and Silica Nanostructure-Based Recovery of Strawberry Plants Subjected to Drought Stress. *Sci. Rep.* **2020**, *10*, 17672. [\[CrossRef\]](#)
11. Rayman, M.P. Selenium in Cancer Prevention: A Review of the Evidence and Mechanism of Action. *Proc. Nutr. Soc.* **2005**, *64*, 527–542. [\[CrossRef\]](#)
12. Zhu, Z.; Chen, Y.; Zhang, X.; Li, M. Effect of Foliar Treatment of Sodium Selenate on Postharvest Decay and Quality of Tomato Fruits. *Sci. Hortic.* **2016**, *198*, 304–310. [\[CrossRef\]](#)
13. Saldaña-Sánchez, W.D.; León-Morales, J.M.; López-Bibiano, Y.; Hernández-Hernández, M.; Langerica-Velázquez, E.C.; García-Morales, S. Effect of V, Se, and Ce on Growth, Photosynthetic Pigments, and Total Phenol Content of Tomato and Pepper Seedlings. *J. Soil Sci. Plant Nutr.* **2019**, *19*, 678–688. [\[CrossRef\]](#)
14. Ramasamy, S.; Nandagopal, J.G.T.; Balasubramanian, M.; Girija, S. Effect of Absciscic Acid and Selenium Foliar Sprays on Drought Mitigation in Tomato (*Solanum lycopersicum* L.). In *Materials Today-Proceedings*; Elsevier: Amsterdam, The Netherlands, 2022; Volume 48, pp. 191–195.
15. Zhong, Y.; Cui, H.; Li, H.; Qiang, X.; Han, Q.; Liu, H. Foliar Application of Selenium Enhances Drought Tolerance in Tomatoes by Modulating the Antioxidative System and Restoring Photosynthesis. *Agronomy* **2024**, *14*, 1184. [\[CrossRef\]](#)
16. Rady, M.M.; Belal, H.E.E.; Gadallah, F.M.; Semida, W.M. Selenium Application in Two Methods Promotes Drought Tolerance in *Solanum lycopersicum* Plant by Inducing the Antioxidant Defense System. *Sci. Hortic.* **2020**, *266*, 109290. [\[CrossRef\]](#)
17. Ragályi, P.; Takács, T.; Füzy, A.; Uzing, N.; Dobosy, P.; Záray, G.; Szűcs-Vásárhelyi, N.; Rékási, M. Effect of Se-Enriched Irrigation Water on the Biomass Production and Elemental Composition of Green Bean, Cabbage, Potato and Tomato. *Plants* **2021**, *10*, 2086. [\[CrossRef\]](#) [\[PubMed\]](#)
18. Dima, S.-O.; Neamtu, C.; Desliu-Avram, M.; Ghiurea, M.; Capra, L.; Radu, E.; Stoica, R.; Faraon, V.-A.; Zamfiropol-Cristea, V.; Constantinescu-Aruxandei, D.; et al. Plant Biostimulant Effects of Baker's Yeast Vinasse and Selenium on Tomatoes Through Foliar Fertilization. *Agronomy* **2020**, *10*, 133. [\[CrossRef\]](#)

19. Germ, M.; Kreft, I.; Stibilj, V.; Urbanc-Berčič, O. Combined Effects of Selenium and Drought on Photosynthesis and Mitochondrial Respiration in Potato. *Plant Physiol. Biochem.* **2007**, *45*, 162–167. [CrossRef]
20. Kostopoulou, P.; Barbayiannis, N.; Noitsakis, B. Water Relations of Yellow Sweetclover Under the Synergy of Drought and Selenium Addition. *Plant Soil.* **2010**, *330*, 65–71. [CrossRef]
21. Meucci, A.; Shiriaev, A.; Rosellini, I.; Malorgio, F.; Pezzarossa, B. Se-Enrichment Pattern, Composition, and Aroma Profile of Ripe Tomatoes After Sodium Selenate Foliar Spraying Performed at Different Plant Developmental Stages. *Plants* **2021**, *10*, 1050. [CrossRef]
22. Villacampa, Y.; Navarro-Gonzalez, J.F.; Reyes, J.A.; Sastre-Vazquez, P. Numerical and Regression Models for the Leaf Area of Tomato Seedlings. In *Ecosystems and Sustainable Development IX*; Marinov, A.M., Brebbia, C.A., Eds.; Wit Press: Southampton, UK, 2013; Volume 175, pp. 3–10.
23. Vacchina, V.; Dumont, J. Total Selenium Quantification in Biological Samples by Inductively Coupled Plasma Mass Spectrometry (ICP-MS). In *Selenoproteins: Methods and Protocols*; Chavatte, L., Ed.; Humana Press Inc.: Totowa, NJ, USA, 2018; Volume 1661, pp. 145–152, ISBN 978-1-4939-7258-6.
24. Chazaux, M.; Schiphorst, C.; Lazzari, G.; Caffarri, S. Precise Estimation of Chlorophyll a, b and Carotenoid Content by Deconvolution of the Absorption Spectrum and New Simultaneous Equations for Chlorophyll Determination. *Plant J.* **2022**, *109*, 1630–1648. [CrossRef]
25. Bates, L.S.; Waldren, R.P.; Teare, I.D. Rapid Determination of Free Proline for Water-Stress Studies. *Plant Soil.* **1973**, *39*, 205–207. [CrossRef]
26. Zhang, Q.; Li, Y.; Sun, X.; Xing, S.; Cong, W.; Liu, X. Study on Dormancy Mechanism and Breaking Dormancy Method of *Viburnum Sargentii* Seeds. *Am. J. Plant Sci.* **2019**, *10*, 65–78. [CrossRef]
27. Li, L.; Wang, S.; Wu, S.; Rao, S.; Li, L.; Cheng, S.; Cheng, H. Morphological and Physiological Indicators and Transcriptome Analyses Reveal the Mechanism of Selenium Multilevel Mitigation of Cadmium Damage in Brassica Juncea. *Plants* **2023**, *12*, 1583. [CrossRef]
28. Kar, R.K. Plant Responses to Water Stress Role of Reactive Oxygen Species. *Plant Signal. Behav.* **2011**, *6*, 1741–1745. [CrossRef]
29. Hasanuzzaman, M.; Bhuyan, M.H.M.B.; Zulfiqar, F.; Raza, A.; Mohsin, S.M.; Al Mahmud, J.; Fujita, M.; Fotopoulos, V. Reactive Oxygen Species and Antioxidant Defense in Plants Under Abiotic Stress: Revisiting the Crucial Role of a Universal Defense Regulator. *Antioxidants* **2020**, *9*, 681. [CrossRef] [PubMed]
30. Hussain, H.A.; Men, S.; Hussain, S.; Chen, Y.; Ali, S.; Zhang, S.; Zhang, K.; Li, Y.; Xu, Q.; Liao, C.; et al. Interactive Effects of Drought and Heat Stresses on Morpho-Physiological Attributes, Yield, Nutrient Uptake and Oxidative Status in Maize Hybrids. *Sci. Rep.* **2019**, *9*, 3890. [CrossRef] [PubMed]
31. Sun, J.; Gu, J.; Zeng, J.; Han, S.; Song, A.; Chen, F.; Fang, W.; Jiang, J.; Chen, S. Changes in Leaf Morphology, Antioxidant Activity and Photosynthesis Capacity in Two Different Drought-Tolerant Cultivars of Chrysanthemum During and After Water Stress. *Sci. Hortic.* **2013**, *161*, 249–258. [CrossRef]
32. Feng, R.; Wei, C.; Tu, S. The Roles of Selenium in Protecting Plants Against Abiotic Stresses. *Environ. Exp. Bot.* **2013**, *87*, 58–68. [CrossRef]
33. Ghorbanli, M.; Gafarabad, M.; Amirkian, T.; Allahverdi, B. Investigation of Proline, Total Protein, Chlorophyll, Ascorbate and Dehydroascorbate Changes Under Drought Stress in Akria and Mobil Tomato Cultivars. Available online: https://journals.iau.ir/article_540675_e99838d78036ac679c11f1e0382758d0.pdf (accessed on 3 September 2025).
34. Khan, R.; Ma, X.; Zhang, J.; Wu, X.; Iqbal, A.; Wu, Y.; Zhou, L.; Wang, S. Circular Drought-Hardening Confers Drought Tolerance via Modulation of the Antioxidant Defense System, Osmoregulation, and Gene Expression in Tobacco. *Physiol. Plant.* **2021**, *172*, 1073–1088. [CrossRef]
35. Neysanian, M.; Iranbakhsh, A.; Ahmadvand, R.; Ardebili, Z.O.; Ebadi, M. Selenium Nanoparticles Conferred Drought Tolerance in Tomato Plants by Altering the Transcription Pattern of microRNA-172 (miR-172), bZIP, and CRTISO Genes, Upregulating the Antioxidant System, and Stimulating Secondary Metabolism. *Protoplasma* **2024**, *261*, 735–747. [CrossRef]
36. Hussain, S.; Ahmed, S.; Akram, W.; Li, G.; Yasin, N.A. Selenium Seed Priming Enhanced the Growth of Salt-Stressed *Brassica rapa* L. Through Improving Plant Nutrition and the Antioxidant System. *Front. Plant Sci.* **2023**, *13*, 1050359. [CrossRef]
37. Hernández-Hernández, H.; Juárez-Maldonado, A.; Benavides-Mendoza, A.; Ortega-Ortiz, H.; Cadenas-Pliego, G.; Sánchez-Aspeytia, D.; González-Morales, S. Chitosan-PVA and Copper Nanoparticles Improve Growth and Overexpress the SOD and JA Genes in Tomato Plants Under Salt Stress. *Agronomy* **2018**, *8*, 175. [CrossRef]
38. Sairam, R.K.; Srivastava, G.C. Water Stress Tolerance of Wheat (*Triticum aestivum* L.): Variations in Hydrogen Peroxide Accumulation and Antioxidant Activity in Tolerant and Susceptible Genotypes. *J. Agron. Crop Sci.* **2001**, *186*, 63–70. [CrossRef]
39. Morales-Espinoza, M.C.; Cadenas-Pliego, G.; Pérez-Alvarez, M.; Hernández-Fuentes, A.D.; Cabrera de la Fuente, M.; Benavides-Mendoza, A.; Valdés-Reyna, J.; Juárez-Maldonado, A. Se Nanoparticles Induce Changes in the Growth, Antioxidant Responses, and Fruit Quality of Tomato Developed Under NaCl Stress. *Molecules* **2019**, *24*, 3030. [CrossRef] [PubMed]

40. Malik, J.A.; Kumar, S.; Thakur, P.; Sharma, S.; Kaur, N.; Kaur, R.; Pathania, D.; Bhandhari, K.; Kaushal, N.; Singh, K.; et al. Promotion of Growth in Mungbean (*Phaseolus aureus* Roxb.) by Selenium Is Associated with Stimulation of Carbohydrate Metabolism. *Biol. Trace Elem. Res.* **2011**, *143*, 530–539. [\[CrossRef\]](#)
41. Lanza, M.G.D.B.; dos Reis, A.R. Roles of Selenium in Mineral Plant Nutrition: ROS Scavenging Responses Against Abiotic Stresses. *Plant Physiol. Biochem.* **2021**, *164*, 27–43. [\[CrossRef\]](#) [\[PubMed\]](#)
42. Yaish, M.W. Proline Accumulation Is a General Response to Abiotic Stress in the Date Palm Tree (*Phoenix dactylifera* L.). *Genet. Mol. Res.* **2015**, *14*, 9943–9950. [\[CrossRef\]](#)
43. Szabados, L.; Savaouré, A. Proline: A Multifunctional Amino Acid. *Trends Plant Sci.* **2010**, *15*, 89–97. [\[CrossRef\]](#) [\[PubMed\]](#)
44. Kishor, P.B.K.; Sangam, S.; Amrutha, R.N.; Laxmi, P.S.; Naidu, K.R.; Rao, K.; Rao, S.; Reddy, K.J.; Theriappan, P.; Sreenivasulu, N. Regulation of Proline Biosynthesis, Degradation, Uptake and Transport in Higher Plants: Its Implications in Plant Growth and Abiotic Stress Tolerance. *Curr. Sci.* **2005**, *88*, 424–438.
45. Verbruggen, N.; Hermans, C. Proline Accumulation in Plants: A Review. *Amino Acids* **2008**, *35*, 753–759. [\[CrossRef\]](#) [\[PubMed\]](#)
46. Sharma, S.; Villamor, J.G.; Verslues, P.E. Essential Role of Tissue-Specific Proline Synthesis and Catabolism in Growth and Redox Balance at Low Water Potential. *Plant Physiol.* **2011**, *157*, 292–304. [\[CrossRef\]](#)
47. Khan, T.A.; Ahmad, A.; Saeed, T.; Yusuf, M.; Faisal, M.; Alatar, A.A. Investigating the Influence of Selenium and Epibrassinolide on Antioxidant Activity, Proline Accumulation, and Protein Expression Profiles in Wheat Plants Experiencing Heat and Drought Stress. *Front. Plant Sci.* **2024**, *15*, 1441483. [\[CrossRef\]](#)
48. Lanza, M.G.D.B.; Silva, V.M.; Montanha, G.S.; Lavres, J.; de Carvalho, H.W.P.; dos Reis, A.R. Assessment of Selenium Spatial Distribution Using μ -XFR in Cowpea (*Vigna unguiculata* (L.) Walp.) Plants: Integration of Physiological and Biochemical Responses. *Ecotox. Environ. Safe.* **2021**, *207*, 111216. [\[CrossRef\]](#)
49. Khan, M.I.R.; Nazir, F.; Asgher, M.; Per, T.S.; Khan, N.A. Selenium and Sulfur Influence Ethylene Formation and Alleviate Cadmium-Induced Oxidative Stress by Improving Proline and Glutathione Production in Wheat. *J. Plant Physiol.* **2015**, *173*, 9–18. [\[CrossRef\]](#)
50. Cheng, H.; Kong, W.; Tang, T.; Ren, K.; Zhang, K.; Wei, H.; Lin, T. Identification of Key Gene Networks Controlling Soluble Sugar and Organic Acid Metabolism During Oriental Melon Fruit Development by Integrated Analysis of Metabolic and Transcriptomic Analyses. *Front. Plant Sci.* **2022**, *13*, 830517. [\[CrossRef\]](#)
51. Nezamivand Chegini, S.; Jafarinia, M.; Ghotbi-Ravandi, A.A. Unraveling the Impacts of Progressive Drought Stress on the Photosynthetic Light Reaction of Tomato: Assessed by Chlorophyll-a Fluorescence and Gene Expression Analysis. *Cell. Mol. Biol.* **2024**, *70*, 176–184. [\[CrossRef\]](#)
52. Tanaka, A.; Tanaka, R. Chlorophyll Metabolism. *Curr. Opin. Plant Biol.* **2006**, *9*, 248–255. [\[CrossRef\]](#)
53. Hayat, S.; Hayat, Q.; Alyemeni, M.N.; Wani, A.S.; Pichtel, J.; Ahmad, A. Role of Proline Under Changing Environments: A Review. *Plant Signal. Behav.* **2012**, *7*, 1456–1466. [\[CrossRef\]](#) [\[PubMed\]](#)
54. Osakabe, Y.; Osakabe, K.; Shinozaki, K.; Tran, L.-S.P. Response of Plants to Water Stress. *Front. Plant Sci.* **2014**, *5*, 86. [\[CrossRef\]](#) [\[PubMed\]](#)
55. Chen, M. Chlorophyll Modifications and Their Spectral Extension in Oxygenic Photosynthesis. In *Annual Review of Biochemistry*; Kornberg, R.D., Ed.; Annual Reviews: Palo Alto, CA, USA, 2014; Volume 83, pp. 317–340. ISBN 978-0-8243-0883-4.
56. Verslues, P.E.; Sharma, S. Proline Metabolism and Its Implications for Plant-Environment Interaction. *Arab. Book/Am. Soc. Plant Biol.* **2010**, *8*, e0140. [\[CrossRef\]](#)
57. Bai, B.; Wang, Z.; Gao, L.; Chen, W.; Shen, Y. Effects of Selenite on the Growth of Alfalfa (*Medicago sativa* L. Cv. Sadie 7) and Related Physiological Mechanisms. *Acta Physiol. Plant* **2019**, *41*, 78. [\[CrossRef\]](#)
58. Zhang, M.; Tang, S.; Huang, X.; Zhang, F.; Pang, Y.; Huang, Q.; Yi, Q. Selenium Uptake, Dynamic Changes in Selenium Content and Its Influence on Photosynthesis and Chlorophyll Fluorescence in Rice (*Oryza sativa* L.). *Environ. Exp. Bot.* **2014**, *107*, 39–45. [\[CrossRef\]](#)
59. Tao, J.; Leng, J.; Lei, X.; Wan, C.; Li, D.; Wu, Y.; Yang, Q.; Wang, P.; Feng, B.; Gao, J. Effects of Selenium (Se) Uptake on Plant Growth and Yield in Common Buckwheat (*Fagopyrum esculentum* moench). *Field Crop. Res.* **2023**, *302*, 109070. [\[CrossRef\]](#)
60. Mackova, J.; Vaskova, M.; Macek, P.; Hronkova, M.; Schreiber, L.; Santrucek, J. Plant Response to Drought Stress Simulated by ABA Application: Changes in Chemical Composition of Cuticular Waxes. *Environ. Exp. Bot.* **2013**, *86*, 70–75. [\[CrossRef\]](#)
61. Zhu, P.; Zhuang, Q.; Ciais, P.; Welp, L.; Li, W.; Xin, Q. Elevated Atmospheric CO₂ Negatively Impacts Photosynthesis Through Radiative Forcing and Physiology-Mediated Climate Feedback. *Geophys. Res. Lett.* **2017**, *44*, 1956–1963. [\[CrossRef\]](#)
62. Cacefo, V.; Ribas, A.F.; Vieira, L.G.E. Proline Metabolism as a Mechanism for the Energy Dissipation in *VaP5CSF129A* Transgenic Tobacco Plants Under Water Deficit. *J. Plant Physiol.* **2023**, *283*, 153964. [\[CrossRef\]](#) [\[PubMed\]](#)

63. De Vita, P.; Platani, C.; Fragasso, M.; Ficco, D.B.M.; Colecchia, S.A.; Del Nobile, M.A.; Padalino, L.; Di Gennaro, S.; Petrozza, A. Selenium-Enriched Durum Wheat Improves the Nutritional Profile of Pasta Without Altering Its Organoleptic Properties. *Food Chem.* **2017**, *214*, 374–382. [[CrossRef](#)] [[PubMed](#)]
64. Nawaz, F.; Ashraf, M.Y.; Ahmad, R.; Waraich, E.A.; Shabbir, R.N.; Hussain, R.A. Selenium Supply Methods and Time of Application Influence Spring Wheat (*Triticum aestivum* L.) Yield Under Water Deficit Conditions. *J. Agric. Sci.* **2017**, *155*, 643–656. [[CrossRef](#)]

Disclaimer/Publisher’s Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.