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Alleviation of Heat Stress in Tomato by Exogenous Application of Sulfur

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Citation: Ali, M.M.; Waleed Shafique, M.; Gull, S.; Afzal Naveed, W.; Javed, T.; Yousef, A.F.; Mauro, R.P. Alleviation of Heat Stress in Tomato by Exogenous Application of Sulfur. *Horticulturae* **2021**, *7*, 21. <https://doi.org/10.3390/horticulturae7020021>

Received: 23 November 2020

Accepted: 25 January 2021

Published: 29 January 2021

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Abstract: Temperature is a key factor influencing plant growth and productivity, however sudden increases in temperature can cause severe consequences in terms of crop performance. We evaluated the influence of elementary sulfur application on the physiology and growth of two tomato genotypes (“Ahmar” and “Roma”) grown in two growth chambers (at 25 and 45 °C). Plants were sprayed with 2, 4, 6, and 8 ppm sulfur 45 days after sowing (untreated plants were kept as control). Plants of the “Roma” cultivar receiving 6 ppm sulfur exhibited maximal shoot and root biomass values followed by those receiving 4 ppm under both temperature conditions. Maximal CO₂ index, photosynthetic rate, transpiration rate, and greenness index values (188.1 μmol mol⁻¹, 36.3 μmol CO₂ m⁻² s⁻¹, 1.8 μmol H₂O m⁻² s⁻¹, and 95 SPAD, respectively) were observed in plants of “Roma” cultivar grown at 25 °C, indicating positive influences of sulfur on tomato physiology. Similarly, sulfur maximized proline, nitrogen, phosphorus, and potassium contents in leaves of both genotypes at both temperatures. The differences between control and sulfur-treated plants grown under heat stress indicate a possible role of sulfur in mitigating heat stress. Overall, our results suggest that 6 ppm of sulfur is the best dose to alleviate tomato heat stress and enhance the morphological, physiological, and biochemical attributes of tomato plants.

Keywords: thermotolerance; abiotic stress; foliar application; *Solanum lycopersicum* L.; SPAD

1. Introduction

Tomato (*Solanum lycopersicum* L.) belongs to the Solanaceae family, which is native to Peruvian and Mexican regions [1,2]. In Pakistan, the various cultivars of tomato are grown over an area of about 58,359 ha, with an annual average production of 550,979 t [3]. Although tomato has good potential to be cultivated in several climatic zones, it faces numerous abiotic stresses [4], of which high temperature is a crucial problem [5].

Tomato productivity is severely influenced by temperature fluctuation [6]. Increases above the optimum temperature hamper certain physiological processes, leading to poor

productivity [7,8]. Heat stress affects several growth phases, i.e., the germination, expansion, and reproduction of plants [9]. High temperature may cause defective performance of the photosynthesis apparatus at the chloroplast level. The carbon metabolism in the stroma and chemical signaling in the thylakoid lamellae have been considered as primary areas of damage due to high temperature [10]. Photosynthesis is more heat-sensitive than dark respiration and is inhibited before the inhibition of respiration due to injury to plants caused by high temperature [11]. High temperature causes water deficiency in plant tissues, which leads to a deficiency of minerals due to lack of transport [12,13]. Tomato cultivars show different responses toward high-temperature stress. For each degree increase above the optimum temperature, yield losses may range up to 10–15% [14].

Technologies and methods need to be introduced to improve the performance of crops under heat stress. Mineral nutrition is the key to regulating plant growth, metabolic functioning, and stress mitigation. Sulfur (S) is considered an important mineral nutrient that is required by the plants during heat stress [15]. It is a major constituent of proteins that are necessary for plant metabolism and is required throughout the plant growth period, from vegetative growth to harvesting [16]. To improve thermotolerance mechanisms, amino acids and metabolites containing S interact with different biological compounds such as plant growth regulators, enzymes, polyamines, and nutrients, and produce certain derivatives that are essential in mitigating heat stress [17]. Sulfur and its derivatives play vital roles in the activation of reactive oxygen species (ROS) scavenging enzymes to improve antioxidant defense under abiotic stresses [18]. Indirectly, S interacts with other molecules, e.g., auxins, cytokinin, gibberellins, ethylene, jasmonic acid, and salicylic acid, to counteract abiotic stresses [19]. Sulfur plays a vital role as a signaling agent in plant cells to allow communication within the cellular environment [20]. Plants need thiol-containing S biomolecules to develop a defensive mechanism against different abiotic stresses [21].

Under controlled conditions, cultivation is quite impractical in Pakistan because of the small landholdings, limited resources, and high energy prices [22]. Furthermore, in traditional cultivation systems, tomato crops face high-temperature stress, leading to poor yield and inferior fruit quality [6]. Hence, investigation is required of the effects of nutrients in mitigating heat stress in tomato plants. Therefore, the present study was conducted to examine the effects of exogenously applied sulfur as a stress-alleviating agent in two different tomato cultivars.

2. Materials and Methods

2.1. Experimental Site and Conditions

An experiment was conducted under controlled conditions at a research farm belonging to the University of Agriculture, Faisalabad, Pakistan (31°25'59.6" N 73°04'18.9" E) from 13 March 2018 to 27 May 2018. Three-month-old seeds of two tomato genotypes, namely "Roma" (thermotolerant) and "Ahmar" (thermosensitive) [23,24], were obtained from the Vegetable Research Institute, Ayyub Agriculture Research Institute, Faisalabad, Punjab, Pakistan. Before sowing, the moisture contents of the seeds were 11% and 10% for "Ahmar" and "Roma", respectively. Seeds were sown in plastic pots (33 × 30 cm) filled with 12 kg porous soil collected from a nearby field. The soil's structural type was sandy loam with electrical conductivity and pH levels of 0.401 dS m⁻¹ and 6.9, respectively. The electric conductivity and pH were recorded with an electrical conductivity (EC) meter (HI-98304, Hanna Instruments Inc., Bedfordshire, UK) and a digital pH meter (Hanna, HI-98107, Mauritius), respectively. Five seeds per pot were sown, and each replication was comprised of five pots. The pots were watered according to the plants' need by observing the moisture of rooting media. Hoagland's solution (0.4 NH₄H₂PO₄; 2.4 KNO₃; 1.6 Ca(NO₃)₂; 0.8 MgSO₄; 0.1 Fe as Fe-chelate; 0.023 B as B(OH)₃ (boric acid); 0.0045 Mn as MnCl₂; 0.0003 Cu as CuCl₂; 0.0015 Zn as ZnCl₂; 0.0001 Mo as MoO₃ or (NH₄)₆Mo₇O₂₄; Cl as

chlorides of Mn, Zn, and Cu (all concentrations in units of $\mu\text{M/L}$) was used for plant fertigation. The experiment was designed under a split-split plot design, having temperature as the main plot factor (2 growth chambers), genotypes as the subplot factors (2 genotypes), and S treatments as sub-subplot factors (5 levels), with four replicates.

2.2. Treatments

Plants of both genotypes were kept in two growth chambers (Jeiotech GC-300TL, Scientific Laboratory Supplies, UK) at an optimum temperature, i.e., 25 °C day/20 °C night, with a light period of 12 h ($100 \pm 2 \mu\text{mol m}^{-2} \text{s}^{-1}$ white florescent light peak wavelength λ_p (544 nm)). Heat treatment was started four weeks after emergence. The temperature of one chamber was increased by 2 °C each day to avoid osmotic shock until the desired temperature level (45 ± 2 °C) was achieved. Relative humidity was maintained during the complete execution of the growth chamber experiment at $65 \pm 5\%$. Different levels of S (CAS no. 43766-A3, $\geq 99.5\%$ purity, Thermo Fisher Scientific, Lancashire, UK) (2, 4, 6, and 8 ppm) were applied twice (15 and 22 days after heat induction) through foliar spray in both growth chambers. Control plants were sprayed with water only.

2.3. Morphological Variables

Morphological variables were measured 30 days after S application. Shoot and root lengths were measured with the help of a meter rod from five randomly selected plants from each replication, then averages were calculated. The fresh weights of shoots and roots were measured using a digital weighing balance (MJ-W176P, Panasonic, Japan). For dry weight determination, shoots and roots were oven-dried at 70 °C (YH-9203A, Qingdao Yosion Labtech Co. Ltd., Qingdao, China) until constant weight was reached [25].

2.4. Physiological Variables

Plant physiological variables, i.e., the CO_2 index ($\mu\text{mol mol}^{-1}$), photosynthetic rate ($\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$), and transpiration rate ($\mu\text{mol H}_2\text{O m}^{-2} \text{ s}^{-1}$), were measured with an LCA-4 infrared gas analyzer (ADC BioScientific Ltd., Hoddesdon, UK) from fully expanded leaves 25 days after S application. The greenness index values of the leaves were measured with a chlorophyll Soil Plant Analysis Development (SPAD) meter (CCM-200 plus, Opti-Sciences, Hudson, NH, USA) according to the manufacturer's instructions and presented as SPAD values.

2.5. Biochemical Variables

Fully expanded, mature, and healthy leaves along with petiole were collected from randomly selected plants from each replicate 25 days after S application. Estimations of nitrogen, phosphorus, and potassium in leaf tissues were carried out using a micro-Kjeldahl apparatus, spectrophotometer, and flame photometer, respectively, as described by Estefan et al. [26]. Proline concentration was determined through the method used by Bates et al. [27] using a spectrophotometer. Fresh leaf tissues (0.5 g) were homogenized in 10 mL of 3% sulfo-salicyclic acid. The 2 mL filtered homogenate was taken in a test tube, 2 mL acid ninhydrin solution was added (1.25 g ninhydrin in 30 mL glacial acetic acid and 20 mL 6 M ortho-phosphoric acid) along with 2 mL of glacial acetic acid, then the mixture was heated for 1 h at about 100 °C. The reaction was finished in an ice bath. The reaction mixture was removed with 10 mL toluene then mixed dynamically by passing an incessant stream of air over the mixture for 1–2 min. Toluene was aspirated from chromophore. The aqueous phase was taken and absorbance were observed at 520 nm using toluene as a blank. The proline concentration was evaluated from a standard curve and analyzed on a fresh weight basis as follows:

$$\text{Proline } (\mu\text{mol g}^{-1}) = \frac{[\text{Proline } (\frac{\mu\text{g}}{\text{ml}}) \times \text{toluene (ml)}] / [115.5 (\frac{\mu\text{g}}{\mu\text{mole}})]}{\text{leaf sample (g)}/5} \quad (1)$$

2.6. Statistical Analysis

A three-way analysis of variance (ANOVA), i.e., 2 temperatures, 2 genotypes, and 5 S levels, was executed. Tukey's honest significance difference (HSD) test was used to compare treatment means (when $p \leq 0.05$) using the statistical software package Statistix 8.1. Variables were further subjected to principal component analysis using XLSTAT version 2018. Correlation coefficient values were determined with Pearson's (r) method.

3. Results

3.1. Roma (Thermotolerant)

3.1.1. Morphological Variables

The results are indicative that the untreated plants under heat stress (45 °C) showed minimal shoot length (14.85 cm) as compared to all other treatments. The exogenous application of S not only mitigated the heat stress but enhanced the shoot lengths of both genotypes. The plants treated with 6 ppm S exhibited maximal shoot lengths at both temperatures (35.3 cm for 25 °C and 38.3 cm 45 °C) (Figure 1a). Similarly, the maximal root length (13.49 cm) was observed in plants treated with 6 ppm S under heat stress (45 °C), followed by the plants at normal temperature (25 °C), indicating that 6 ppm S not only induced thermotolerance but increased the below-ground biomass production of tomato plants (Figure 1b).

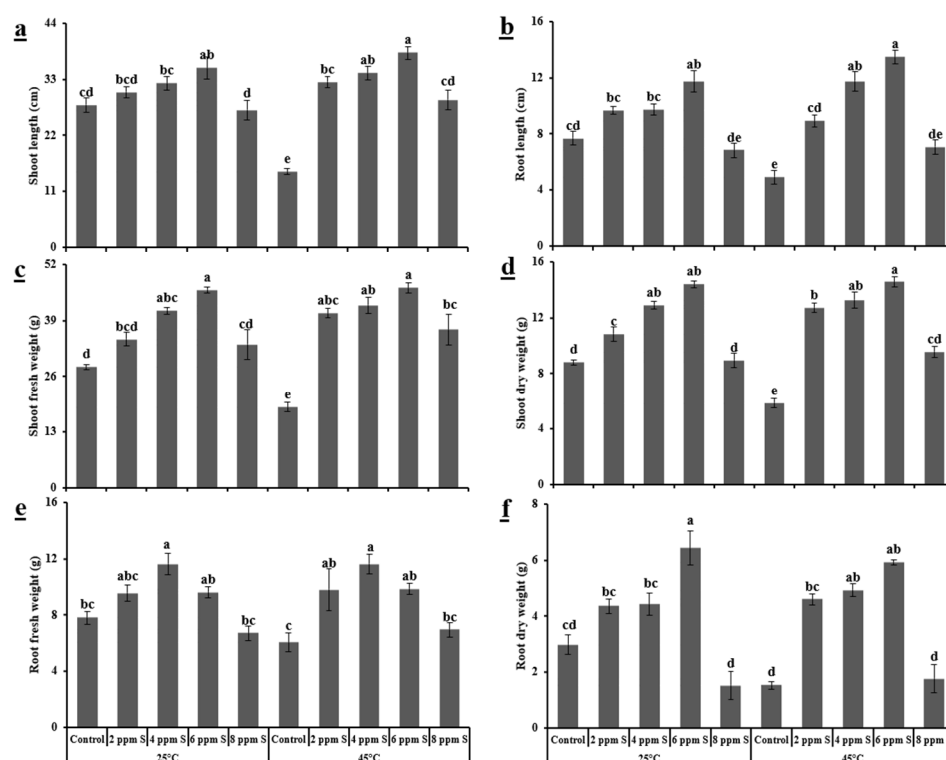


Figure 1. Morphological variables [i.e., shoot length (a), root length (b), shoot fresh weight (c), shoot dry weight (d), root fresh weight (e), root dry weight (f)] of tomato plants (cv. Roma) as affected by temperature and exogenous application of S. Same letters indicate non-significant differences among treatments according to Tukey's honestly significant difference test ($p \leq 0.05$). Vertical bars indicate averages \pm standard errors ($n = 4$, 5 plants per replicate).

Regarding the shoot fresh weight of tomato plants, the plants treated with 6 ppm S showed maximal values in both temperature conditions, followed by 4, 2, and 8 ppm S application groups. Tomato plants under heat stress (45 °C) exhibited minimal shoot fresh weight values until S was applied. The plants treated with 6 ppm S exhibited maximal shoot fresh weight values at 45 °C (46.65 g), as well as at 25 °C (46.1 g) (Figure 1c). A

similar trend was observed with respect to shoot dry weight values. The plants receiving 6 ppm S showed maximal values under both temperature conditions, followed by 4, 2, and 8 ppm S application groups. The plants treated with 6 ppm S exhibited maximal shoot dry weight values at 45 °C (14.57 g), as well as at 25 °C (14.40 g) (Figure 1d). Similar to the aforementioned variables, the highest average root fresh weight (12.21 g) was observed in tomato plants treated with 6 ppm S (Figure 1e). Plants receiving foliar application of 4 ppm S also showed remarkable performance under both temperature conditions. Regarding root dry weight values, the maximal average value (6.44 g) was recorded in plants treated with 6 ppm S under normal temperature conditions (25 °C) (Figure 1f).

3.1.2. Physiological Variables

The tomato plants treated with 6 ppm S showed maximal CO₂ index, photosynthetic rate, transpiration rate, and greenness index values (188.1 $\mu\text{mol mol}^{-1}$, 36.3 $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$, 1.8 $\mu\text{mol H}_2\text{O m}^{-2} \text{ s}^{-1}$, and 95 SPAD, respectively) under normal temperature conditions (25 °C) (Figure 2). Under heat stress conditions, tomato plants receiving 6 ppm S exhibited a 1.9-fold increase in CO₂ index values as compared to control (Figure 2a). Similarly, application of 6 ppm S significantly increased the photosynthetic rate (32.2 $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$) as compared to control (21.6 $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$) (Figure 2b).

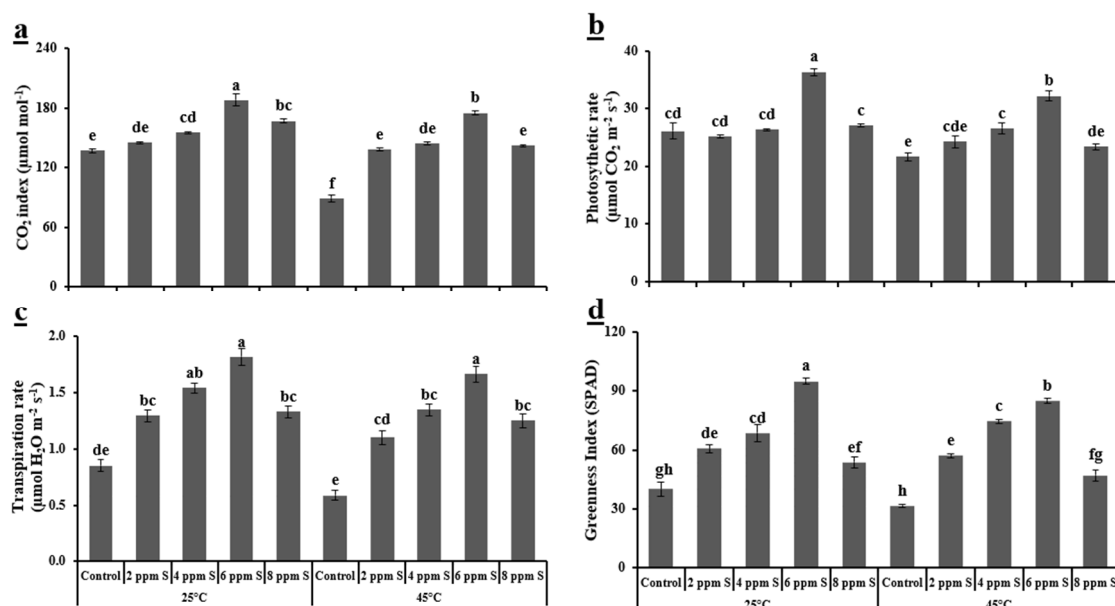


Figure 2. Physiological variables [i.e., CO₂ index (a), photosynthetic rate (b), transpiration rate (c), greenness index (d)] of tomato plants (cv. Roma) as affected by temperature and exogenous application of S. Same letters indicate non-significant differences among treatments according to Tukey's honestly significant difference test ($p \leq 0.05$). Vertical bars indicate averages \pm standard errors ($n = 4, 5$ plants per replicate).

The transpiration rate significantly increased with the foliar application of S. In both growth chambers, the maximal transpiration rates were exhibited by the plants treated with 6 ppm S (1.8 $\mu\text{mol H}_2\text{O m}^{-2} \text{ s}^{-1}$ at 25 °C and 1.7 $\mu\text{mol H}_2\text{O m}^{-2} \text{ s}^{-1}$ 45 °C) (Figure 2c). Similarly, plants receiving foliar application of 6 ppm S showed 137% and 168% increases in greenness index values at 25 °C and 45 °C, respectively (Figure 2d).

3.1.3. Biochemical Variables

Tomato plants treated with 6 ppm S showed maximal leaf proline contents (24.8 $\mu\text{mol g}^{-1}$) under normal temperature (25 °C) conditions, followed by heat stress (45 °C). The leaf proline contents increased with S application in a dose-dependent manner under heat stress (Figure 3).

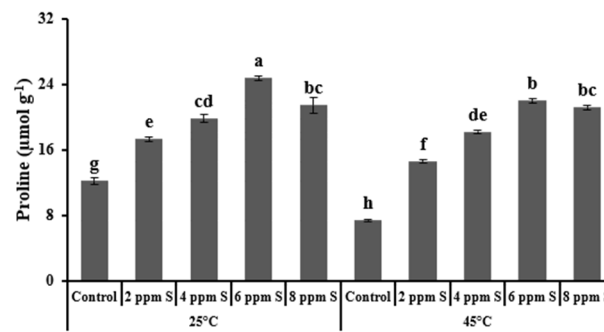


Figure 3. Proline contents of tomato plants (cv. Roma) as affected by temperature and exogenous application of S. Same letters indicate non-significant differences among treatments according to Tukey's honestly significant difference test ($p \leq 0.05$). Vertical bars indicate averages \pm standard errors ($n = 4$, 5 plants per replicate).

Similarly, tomato plants treated with 6 ppm S showed maximal leaf contents of nitrogen, phosphorus, and potassium (6.4%, 6%, and 7.4%, respectively) under normal temperature conditions (25 °C), followed by heat stress conditions (45 °C). Regarding N levels, plants from both growth chambers showed non-significant differences amongst each other, except the plants not treated with S under heat stress conditions (Figure 4a). Plants treated with 6 ppm S exhibited 2.8- and 4.3-fold increases in leaf P contents under 25 °C and 45 °C conditions, respectively (Figure 4b). Similarly, K levels significantly increased with the exogenous application of S under both temperature conditions (Figure 4c).

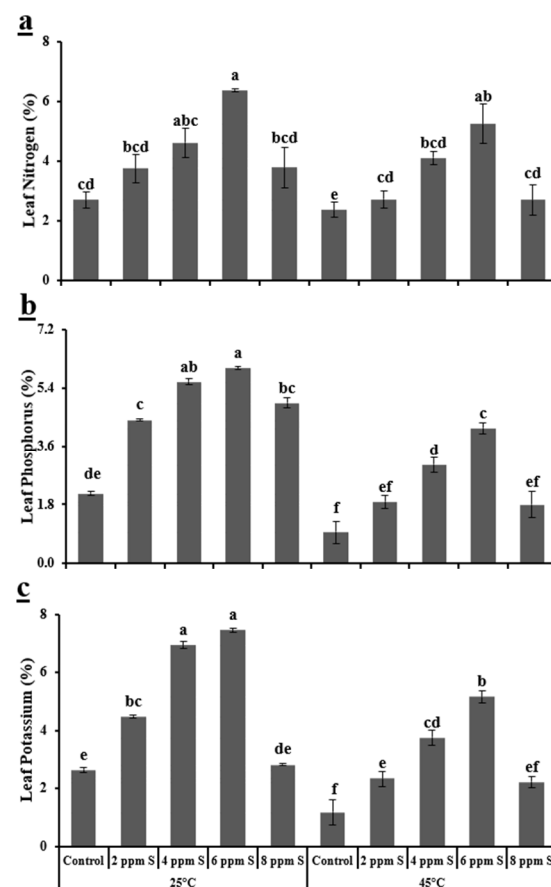


Figure 4. Leaf mineral concentrations [i.e., Nitrogen (a), Phosphorus (b), Potassium (c)] of tomato plants (cv. Roma) as affected by temperature and exogenous application of S. Same letters indicate

non-significant differences among treatments according to Tukey's honestly significant difference test ($p \leq 0.05$). Vertical bars indicate averages \pm standard errors ($n = 4$, 5 plants per replicate).

3.2. Ahmar (Thermotolerant)

3.2.1. Morphological Variables

The results indicate that the plants not treated with S receiving heat stress (45 °C) showed minimal shoot lengths (8.37 cm) as compared to all other treatments. The exogenous application of S not only mitigated the heat stress but enhanced the shoot length values of tomato plants. The plants treated with 6 ppm S exhibited maximal shoot length values under both temperature conditions (Figure 5a). Similarly, maximal root length values (12.3 cm) were observed in plants treated with 6 ppm S under heat stress (45 °C), followed by the plants receiving normal temperature (25 °C) (Figure 5b).

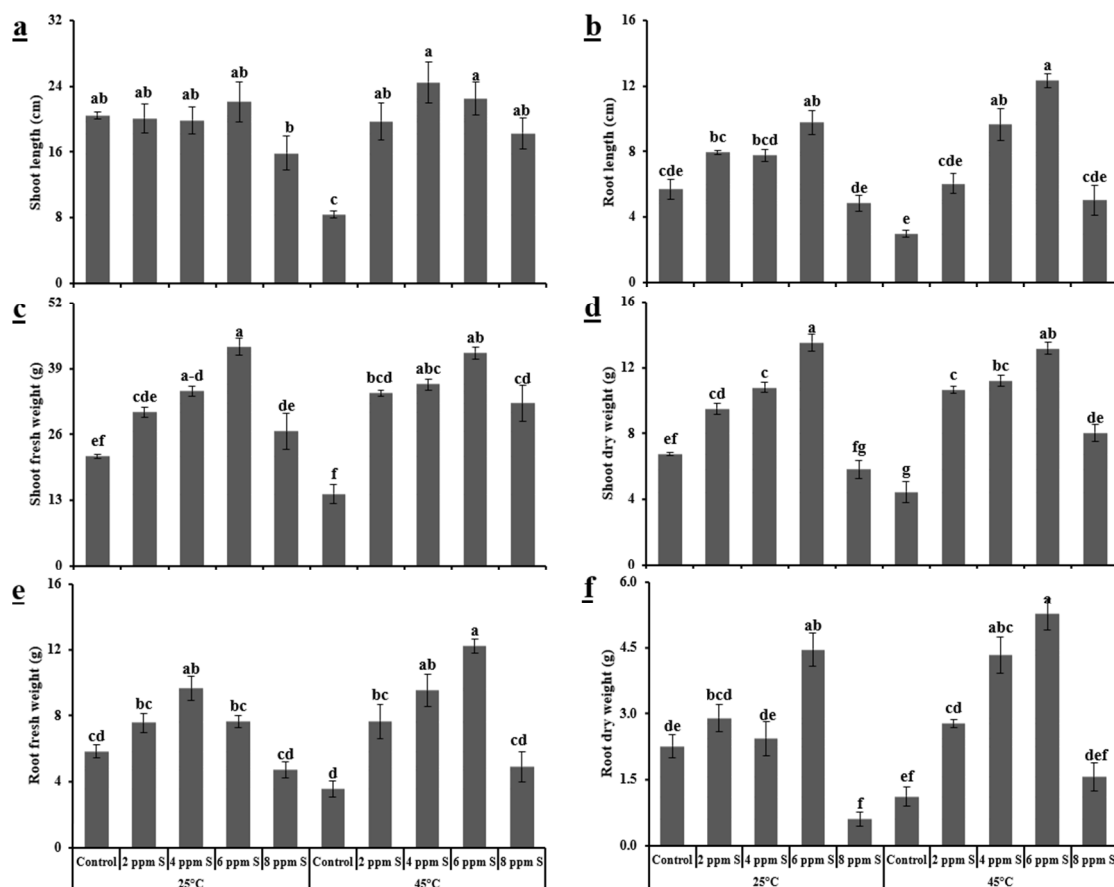


Figure 5. Morphological variables [i.e., shoot length (a), root length (b), shoot fresh weight (c), shoot dry weight (d), root fresh weight (e), root dry weight (f)] of tomato plants (cv. Ahmar) as affected by temperature and exogenous application of S. Same letters indicate non-significant differences among treatments according to Tukey's honestly significant difference test ($p \leq 0.05$). Vertical bars indicate averages \pm standard errors ($n = 4$, 5 plants per replicate).

Ahmar, being a thermosensitive cultivar, exhibited poor results compared to Roma in terms of biomass accumulation. Regarding the shoot fresh weight of tomato plants, the plants treated with 6 ppm S showed maximal values under both temperature conditions (43.3 g at 25 °C and 42.1 g at 45 °C), followed by 4, 2, and 8 ppm S application groups. Tomato plants receiving heat stress (45 °C) exhibited minimal shoot fresh weight values until S was applied. The plants treated with 6 ppm S exhibited maximal shoot fresh weight values at 45 °C (42.14 g) as well as 25 °C (43.32 g) (Figure 5c). A similar trend was observed with respect to shoot dry weight values. The plants receiving 6 ppm S showed maximal values under both temperature conditions, followed by 4, 2, and 8 ppm S application groups. The plants treated with 6 ppm S exhibited maximal shoot dry weight values at 45

°C (13.16 g) as well as at 25 °C (13.53 g) (Figure 5d). Similar to the aforementioned variables, the highest average value for root fresh weight (12.21 g) was observed in tomato plants treated with 6 ppm S (Figure 5e). Regarding root dry weight values, the maximal average value (5.3 g) was recorded in plants treated with 6 ppm S under normal temperature (25 °C) (Figure 5f).

3.2.2. Physiological Variables

Regardless of concentration, CO₂ index values increased with the exogenous application of S when applied on tomato plants under both temperature conditions (Figure 6a). Ahmar, being a thermosensitive cultivar, showed a 2.2-fold decrease in the photosynthetic rate of control plants under heat stress as compared to plants grown under optimal temperature. However, the application of 6 ppm S significantly increased the photosynthetic rate (21.9 $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$) as compared to control (5.5 $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$) (Figure 6b).

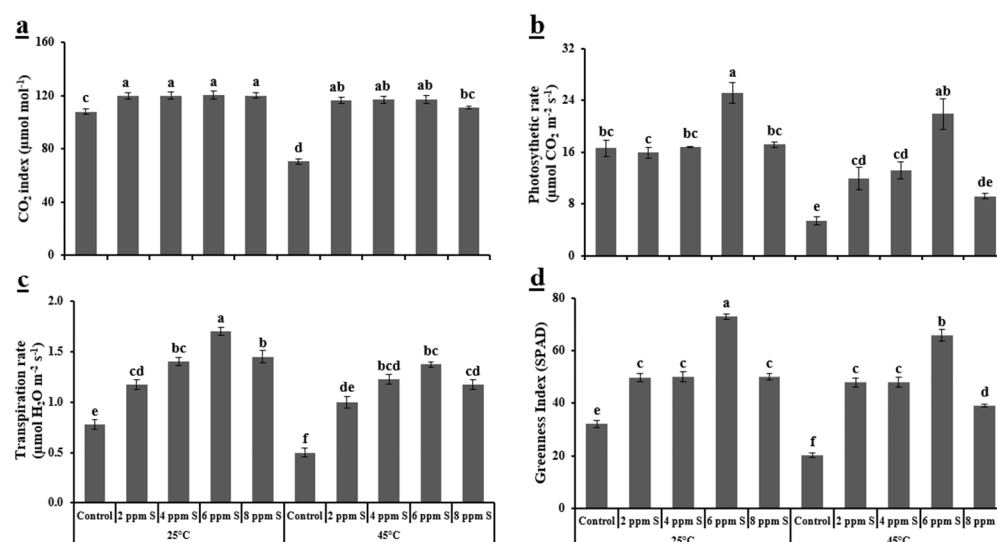


Figure 6. Physiological variables [i.e., CO₂ index (a), photosynthetic rate (b), transpiration rate (c), greenness index (d)] of tomato plants (cv. Ahmar) as affected by temperature and exogenous application of S. Same letters indicate non-significant differences among treatments according to Tukey's honestly significant difference test ($p \leq 0.05$). Vertical bars indicate averages \pm standard errors ($n = 4, 5$ plants per replicate).

The transpiration rates of tomato plants significantly increased with the foliar application of S. In both growth chambers, the maximal transpiration rate was exhibited by the plants treated with 6 ppm S (1.7 $\mu\text{mol H}_2\text{O m}^{-2} \text{ s}^{-1}$ '25 °C', 1.4 $\mu\text{mol H}_2\text{O m}^{-2} \text{ s}^{-1}$ '45 °C) (Figure 6c). Similarly, plants receiving the same foliar application showed 123% and 219% increases in greenness index values at 25 °C and 45 °C, respectively. Although Ahmar is a heat-sensitive cultivar, it performed better when subjected to the exogenous application of S (Figure 6d).

3.2.3. Biochemical Variables

Tomato plants treated with 6 ppm S showed maximal leaf proline content values (19 $\mu\text{mol g}^{-1}$) under normal temperature conditions (25 °C), followed by heat stress conditions (45 °C). Leaf proline contents increased with S application in a dose-dependent manner under heat stress (Figure 7).

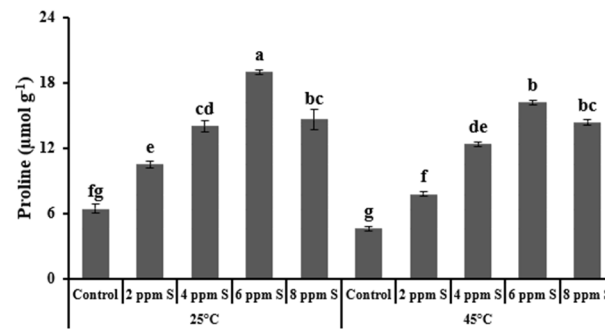


Figure 7. Proline contents of tomato plants (cv. Ahmar) as affected by temperature, genotype, and exogenous application of sulfur. Same letters indicate non-significant differences among treatments according to Tukey's honestly significant difference test ($p \leq 0.05$). Vertical bars indicate averages \pm standard errors ($n = 4, 5$ plants per replicate).

Similarly, tomato plants treated with 6 ppm S showed maximal leaf contents for nitrogen, phosphorus, and potassium (5.4%, 5.6%, and 7%, respectively) under normal temperature conditions (25 °C), followed by heat stress conditions (45 °C) (Figure 8). Regarding leaf N levels, plants from both growth chambers showed non-significant differences among each other, except the plants not treated with S under heat stress conditions (Figure 8a). Plants treated with 6 ppm S exhibited 2.8- and 6.2-fold increases in leaf P contents under 25 °C and 45 °C conditions, respectively (Figure 8b). Ahmar, being a thermosensitive cultivar, showed 3.3- and 3.5-fold decreases in phosphorus and potassium levels, respectively, under heat stress conditions as compared to the plants grown under normal temperature conditions (Figure 8b,c).

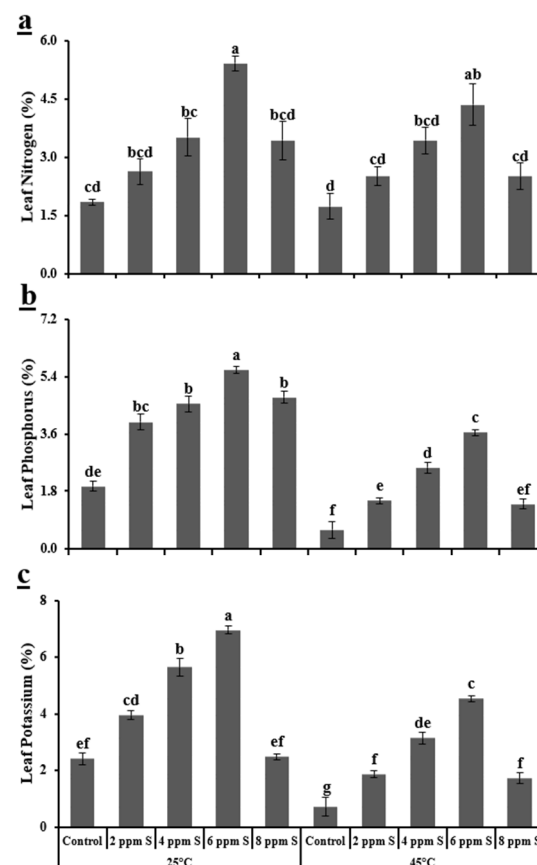


Figure 8. Leaf mineral concentrations [i.e., Nitrogen (a), Phosphorus (b), Potassium (c)] of tomato plants (cv. Ahmar) as affected by temperature, genotype, and exogenous application of sulfur. Same letters indicate non-significant differences among treatments according to Tukey's honestly

significant difference test ($p \leq 0.05$). Vertical bars indicate averages \pm standard errors ($n = 4, 5$ plants per replicate).

3.3. Principle Component Analysis

Principal component analysis was conducted to demarcate the effects of sulfur (Figure 9). Based on the highest squared cosine values corresponding to factors F1, F2, and F3, morphological, physiological, and biochemical variables were clustered with 6 ppm the S treatment, followed by 4, 2, and 8 ppm groups. Factor F1, covering 76.1% of the variability in data (eigenvalue: 10.655), showed clustering of all independent variables with 6 ppm S treatment under both temperature conditions (25 and 45 °C), suggesting a positive influence of S application on these parameters. The second factor covered 9.13% variability in data (eigenvalue: 1.279) and did not show clustering of any dependent variable. The third factor of the principal component analysis covered 6.77% variability in data (eigenvalue: 0.948).

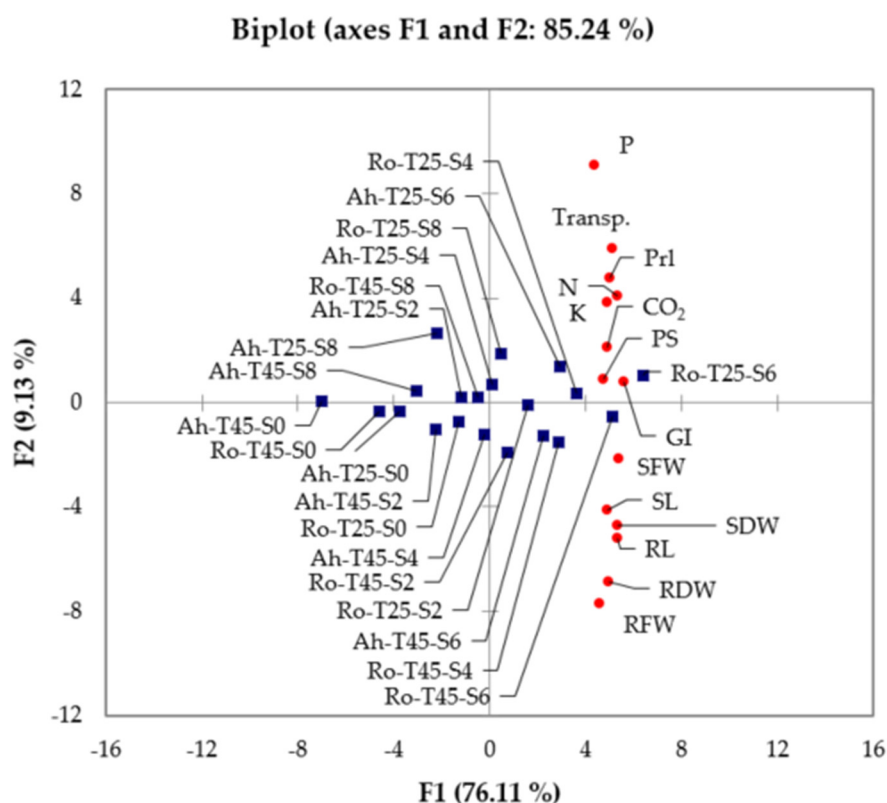


Figure 9. Principal component analysis of sulfur treatments and various morphological, physiological, and biochemical variables of tomato cultivars Ahmad and Roma. Abbreviations: Ah—Ahmar; Ro—Roma; T25—temperature 25 °C; T45—temperature 45 °C; S0—control; S2—2 ppm S; S4—4 ppm S; S6—6 ppm S; S8—8 ppm S; SL—shoot length; RL—root length; SFW—shoot fresh weight; SDW—shoot dry weight; RFW—root fresh weight; RDW—root dry weight; CO₂—CO₂ index; PS—photosynthetic rate; Transp.—transpiration rate; GI—greenness index; Prl—leaf proline content; N—leaf nitrogen content; P—leaf phosphorus content; K—leaf potassium content.

4. Discussion

4.1. Roma (*Thermotolerant*)

High temperature influences plant growth and development in many ways. Plant biomass accumulation is regulated by the movement of cyclin-dependent kinase enzyme, which is reduced due to increases in temperature [28]. The present study confirmed that the lengths and fresh and dry weights of shoots and roots were severely affected by heat stress. However, the cultivar Roma was not affected much and sustained its biomass

(Figure 1). Sulfur (a stress-mitigating agent) was applied through foliar application to mitigate the heat stress of tomato plants. Plants treated with 6 ppm S exhibited maximal biomass accumulation as compared to other S treatments and control (Figure 1), indicating the positive role of S in enhancing plant growth and mitigating the effect of heat stress. Our results are in line with the study by Orman and Kaplan [29], who reported that S application increased the biomass of tomato plants grown in sandy loam soil by 6–8%.

Fluctuations in temperature severely hamper the physiological and developmental attributes of tomato [30]. Decreases in the photosynthetic rate interfere with the function of mitochondria and are the major cause of reductions in plant growth [31]. In our study, as the result of heat induction, the net photosynthesis rate of the tomato plants decreased as compared to the plants grown under normal temperature conditions (Figure 2b). During photosynthesis, Rubisco formation (Calvin cycle) is considered a critical step, which is repressed at 35–40 °C, resulting in decreased net photosynthesis adaptation and production of carbohydrates [32]. Plants grown under heat stress at 45 °C showed minimal CO₂ index values as compared to those grown under normal temperature conditions and with foliar spray with S (Figure 2a).

Heat stress suddenly boosts the rate of transpiration and causes organ dehydration and limited growth [33,34]. It also disturbs the uptake and translocation of water, ions, and whole solutes across the plant membranes and affects photosynthesis and transpiration rates [35]. When the photosynthesis rate is decreased, inhibition of photosystem II (PSII) leads to the breakdown of chlorophyll pigmentation [36,37]. The greenness index values of tomato leaves were also reduced as a result of heat stress (Figure 2d). In the present experiment, leaf nitrogen, phosphorus, potassium, and proline contents were reduced by the heat stress, while plants receiving foliar application of S not only sustained but increased their nutrition levels (Figures 3 and 4). Sulfur application significantly increased the absorption of nitrogen in wheat and canola [38].

4.2. Ahmar (*Thermosensitive*)

The present study revealed that the heat stress severely affected the morphological growth of tomato (cv. Ahmar). Ahmar, being a thermosensitive cultivar, showed less biomass accumulation under heat stress conditions (Figure 5). Increases in senescence due to high temperature is another reason behind reduced accumulation of biomass. Maize and wheat plants exhibited less biomass and reduced yield due to increased senescence at high temperatures [39,40]. Tomato plants treated with 6 ppm S attained maximal biomass levels as compared to other treatments, including control (Figure 5). Our results are in line with the study by Moreira et al. [41], who reported that gypsum (a source of sulfur) application increased the mowing frequency of alfalfa plants, indicating a significant increase in dry matter production. The average shoot dry mass of tomato plants increased by 77% following the application of 100 mg kg⁻¹ S as compared to control [42].

Regarding physiological variables, the net photosynthesis rate and CO₂ index values for tomato plants (cv. Ahmar) were severely hampered under the influence of heat stress (Figure 6). Our study results are in agreement with another study reporting heat stress severely damaged mesophyll cells and increased the permeability of the plasma membrane, resulting in decreased stomatal conductance in grapes [43]. Browning of leaves and stems, stunted growth, leaf abscission, and short lengths of roots and shoots are the macroscopic evidence of physiological damage under heat stress [44,45]. According to our findings, the plants receiving heat stress exhibited reduced greenness index values as compared to the plants grown under normal temperature conditions (Figure 6d). Heat stress may disrupt the thylakoid membrane, causing a decrease in chlorophyll content [46]. Sufficient S nourishment to the plants improves photosynthesis by increasing chlorophyll formation and contributes to the growth and development of plants [47]; moreover, it severely influences the activity of xylem and phloem by decreasing mineral transport in tomato plants [48].

Sulfur has a synergistic relationship with nitrogen, phosphorus, and potassium, and promotes plant growth via maximal uptake of these nutrients [42]. Furthermore, it has an insightful relation with N assimilation. As shown in Figures 7 and 8, tomato plants receiving heat stress exhibited reduced leaf proline, N, P, and K contents, while plants treated with S not only sustained but increased their nutrition levels (Figures 7 and 8). Changes in the levels of mineral nutrients are directly related to changes in the physiological responses of plants [49]. Carciochi et al. [50] reported that optimum S in the growing media of wheat (Z51) increased nitrogen uptake and improved root growth, playing a central role in improving yield [51].

The efficacy of S to modulate plant physiology depends on its concentration, application method, and plant genetics [52]. The results from this study also showed that the tomato plant growth and development responses to S application changed with changes in the concentration of S. Thus, principal component analysis helped to determine the individual roles of S concentrations in regulating various physiological aspects of tomato growth. Overall, the results suggested that S promoted the growth of tomato plants under heat stress. Foliar application of 6 ppm S was positively correlated with the morphological, physiological, and biochemical attributes of tomato.

5. Conclusions

This study suggests that foliar application of sulfur can be used to mitigate heat stress and increase the physiological responses and growth of tomato plants. Since foliar applications of 2, 4, 6, and 8 ppm S differentially regulate distinct aspects of plant growth and development, specific concentrations of S may help achieve specific thermotolerance objectives. Overall, 6 ppm S may be used as an effective exogenous application strategy to improve the health of tomato plants in cases of heat stress. There is a need to investigate S-induced molecular mechanisms regulating stress-related aspects.

Author Contributions: Conceptualization, M.M.A. and M.W.S.; methodology, M.W.S.; data curation, S.G. and R.P.M.; writing—original draft preparation, M.M.A. and W.A.N.; writing—review and editing, M.W.S., S.G., T.J., A.F.Y., and R.P.M. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Acknowledgments: The authors would like to acknowledge the efforts of laboratory and field staff of the Institute of Soil and Environmental Sciences, University of Agriculture, Faisalabad, Punjab, Pakistan.

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

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