Physiological, Morphological and Biochemical Responses of Exogenous Hydrogen Sulfide in Salt-Stressed Tomato Seedlings

Ertan Yildirim 1, Melek Ekinci 1,*, Metin Turan 2, Selda Ors 3 and Atilla Dursun 1,4

1 Department of Horticulture, Faculty of Agriculture, Atatürk University, Erzurum 25240, Turkey
2 Department of Agricultural Trade and Management, Faculty of Economy and Administrative Sciences, Yeditepe University, Istanbul 34755, Turkey
3 Department of Agricultural Structures and Irrigation, Faculty of Agriculture, Atatürk University, Erzurum 25240, Turkey
4 Department of Horticulture and Agronomy, Faculty of Agriculture, Kyrgyz-Turkish Manas University, Bishkek 720038, Kyrgyzstan

* Correspondence: ekincim@atauni.edu.tr

Abstract: Salinity causes yield and quality losses in agricultural production and therefore great economic losses around the world. Hydrogen sulfide (H\textsubscript{2}S) is known to play a crucial role to ease physiological and metabolic processes in plants, and also increases the tolerance of the plant against many abiotic stress conditions. In this study, we investigated the effects of H\textsubscript{2}S treatments (0, 25, 50, 75 and 100 \muM NaHS were applied as H\textsubscript{2}S donor) to the tomato seedlings to alleviate the harmful effects of salt stress (0, 75 and 150 mM NaCl). There was a significant decrease in plant growth and development in parallel with the increased salt level. Visible changes in plant development were observed after the dose of 75 mM NaCl in the tomato seedling. The effects of different doses of exogenous H\textsubscript{2}S treatment were found to be significant. H\textsubscript{2}S treatment increased the stress tolerance in tomato seedlings by arranging the mineral element and hormone content. Furthermore, H\textsubscript{2}S relieved the effect of stress in plants by increasing photosynthetic activity (photosynthesis rate (P\textsubscript{n}), transpiration rate (T\textsubscript{r}), stomatal conductivity (g\textsubscript{s}) and intercellular CO\textsubscript{2} concentration (C\textsubscript{i})) of the plant. In addition, the effect of H\textsubscript{2}S on salt stress tolerance in tomato seedlings may be due to its positive effect on mineral element contents. As a result, based on the beneficial effects of H\textsubscript{2}S in tomato seedlings under salt stress, this treatment can be considered as an alternative resilience method for cultivation in saline soils or irrigation with low quality waters.

Keywords: enzyme; hormone; mineral content; photosynthesis; salinity; tolerance

1. Introduction

Plants may encounter many negative factors during their growth and development stages. Abiotic stress factors, which are defined as unsuitable environmental and soil factors, are one of the biggest causes of yield and quality losses in agricultural production. Salinity is one of the important abiotic stresses that negatively affect agricultural production in approximately 20% of the irrigated areas in the world [1–3].

The first effect of salinity on plants is the closure of stomata and the inhibition of leaf expansion by causing the osmotic potential in the rhizosphere [4,5]. The second effect is that the ions reach toxic levels in a longer period and pile up, especially in mature leaves, and cause the early aging of the leaves, resulting in a decrease in yield and the death of the plant [6]. The high concentration of Na ions has a toxic effect on cell metabolism and inhibits enzyme activity, cell division and expansion, causes membrane irregularity and osmotic imbalance and inhibits growth. Salt stress also causes the production of reactive oxygen species (ROS) and decreased photosynthesis [2]. Salt stress affects all important processes in plants such as germination, growth, photosynthesis, water relationship, nutrient imbalance and yield [7–13].
Studies on salinity stress have been carried out on different plants by many researchers to date, and the tolerance levels of plants to salinity have been tried to be determined. In some of the studies, it has been determined that some substances (hormones, nitric oxide, etc.) applied to plants can mitigate the damage caused by salt stress on the plant [14–16]. In this sense, H$_2$S has been the issue of many studies in recent years. H$_2$S is known as a colorless and foul-smelling toxic gas which comes from industrial processes [17–23]. H$_2$S is involved in various processes in plants such as germination, stomatal movement, the regulation of senescence in plant organs, photosynthesis, enzyme production and fruit storage [24–29]. Exogenous sodium hydrosulfide (NaHS) treatment as an H$_2$S donor has been shown to be effective in seed germination, organogenesis, lateral root formation and stomatal movement [24,30]. Studies have determined that H$_2$S alleviates the unfavorable effects of many abiotic stress factors in the plant [22,23,31–33]. It has been stated that H$_2$S joins in salt, drought and high temperature tolerance by altering ROS accumulation and regulating transcription in multiple defense-related genetic trails [25]. It has previously been found that the exogenous application of H$_2$S at certain concentrations to the plant reduces the damage of salt stress significantly [17–21].

Some vegetable species and varieties are highly sensitive to abiotic stress factors. Especially in the seedling period, large yield losses may occur due to the low quality of the water used. Tomatoes are considered moderately salt tolerant; the threshold EC values at which 50% yield decline occurs are between 1.7 and 5.0 dS m$^{-1}$ [34,35]. In another study, it was determined that yield losses started with 2–3 dS m$^{-1}$, and a 50% decrease in yield was observed with approximately 9 dS m$^{-1}$. It has been stated that root growth in tomato decreases when salinity reaches 4–6 dS m$^{-1}$ and root growth is less affected by salinity than stem growth [36]. Increased Na content in the roots and leaves of the tomato plant changes the nutrient balance [36,37].

Tomato is one of the most produced vegetables in the world. However, there are a limited number of studies that investigate the H$_2$S treatment on plant growth of tomato under salt stress. In this study, we aimed to investigate the effect of H$_2$S on reducing salt damage in the morphological, physiological and biochemical features of tomato during the seedling period.

2. Materials and Methods

2.1. Plant Materials and Experimental Set-Up

In the study, tomato (Solanum lycopersicum L. cv. H2274) seeds were used as plant material. Irrigation waters consisted of 0, 75 and 150 mM NaCl for salinity treatments and 0, 25, 50, 75 and 100 µM NaHS were applied as H$_2$S donors during the tomato seedling period. The study was carried out in pots in a greenhouse (temperature; 25 ± 2°C/18 ± 2°C day/night, humidity 40 ± 5%). Tomato seeds were first sown in a peat:perlite mixture in 45-well viors. Then, healthy seedlings with 2–3 true leaves were transferred to 2.5 L pots with one seedling in each pot. Soil:peat:sand (3:1:1) mixture was used as plant growing medium. Fertilization was done with 15-15-15 compound fertilizer. Some characteristics of the medium are as follows: pH 7.05, EC 89.5 mikromhos cm$^{-1}$, lime 1.73%, organic matter 1.52%, total N 0.05%, P 39.03 ppm, K 2.61 cmol kg$^{-1}$, Mg 13.56 cmol kg$^{-1}$, Na 2.47 cmol kg$^{-1}$, B 0.04 ppm, Cu 0.92 ppm, Fe 0.62 ppm, Zn 0.55 ppm and Mn 0.14 ppm. Pots were placed according to the randomized plots trial design. There were 180 plants (three replications and four plants in each replicate) in the experiment, which was designed according to completely randomized design. The study was completed 30 days after first saline water irrigation.

2.2. H$_2$S Treatment

After the tomato seedlings were planted in pots, Tween-20 was added to the NaHS (H$_2$S donor) solutions prepared with distilled water at doses of 0, 25, 50, 75 and 100 µM, and sprayed to the leaves at one-week intervals. Treatments were repeated three times.
2.3. Salinity Treatment

Solutions of 0, 75 and 150 mM NaCl were used as irrigation water to create salt stress. Saltwater applications were started two weeks after the tomato seedlings were planted in the pots. Salt stress was gradually increased starting with 25 mM initially and balanced at the main doses determined.

2.4. Analysis and Measurements

2.4.1. Plant Growth Parameters

In this study, plant height, stem diameter, number of leaves, plant fresh and dry weight, root fresh and dry weight were determined at the end of study. The chlorophyll reading value of leaves was determined by the SPAD-502 device (SPAD-502, Konica Minolta Sensing, Inc., Tokyo, Japan). Leaf area in a plant was detected with device of CI-202 Portable area meter (CID, Inc., Camas, WA, USA).

2.4.2. Chlorophyll Content

Chlorophyll content of plants was determined spectrophotometrically at 645 and 663 nm wavelengths by Macedo et al. [38] as mg g$^{-1}$ fresh weight [39]. Chlorophyll calculations were made according to the formula below. V: extraction volume, W: sample weight.

\[
\text{Chlo-a (mg g}^{-1}\text{)} = (12.7 \times 663 \text{ nm}) - (2.69 \times 645 \text{ nm}) \times \frac{V}{W} \times 100
\]

\[
\text{Chlo-b (mg g}^{-1}\text{)} = (22.91 \times 645 \text{ nm}) - (4.68 \times 663 \text{ nm}) \times \frac{V}{W} \times 100
\]

Total Chlo (mg g$^{-1}$) = Chlo-a + Chlo-b

2.4.3. Leaf Relative Water Content (LRWC), Electrolyte Leakage (EL) and Photosynthetic Properties

For LRWC analysis, leaf samples from two plants for each treatment were weighed (FW). Then, water was added, and after waiting for 24 h, the turgor weight of the leaf samples were determined (TW). After this, the samples were dried at 70 $^\circ$C for 48 h and their dry weights (DW) were determined. LRWC was calculated according to Turan et al. [17].

Electrolyte leakage (EL) was determined on leaf samples taken from the middle leaves of two seedlings. Firstly, the samples were incubated for 24 h into distilled water and EL1 was measured with electrical conductivity meter. After the samples were autoclaved at 120 $^\circ$C for 20 min, EL2 was determined when the solution temperature reached 25 $^\circ$C. EL value was defined according to EL1/EL2 $\times$ 100 formula [12,17].

Photosynthesis rate (Pn), transpiration rate (Tr), stomatal conductivity (gs) and intercellular CO$_2$ concentration (Ci) were made from 09:30 to 11:30 h a few days before the harvest with photosynthesis system device (Li6400xt, Li-COR, Lincoln, NE, USA) [40].

2.4.4. Hydrogen Peroxide (H$_2$O$_2$), Malondialdehyde (MDA), Sucrose and Proline Content

H$_2$O$_2$ and MDA content of leaf tissues were determined according to method of Liu et al. [41]. Approximately 0.3 g of fresh leaf samples were homogenized in a mortar with 5% trichloroacetic acid (TCA) and centrifuged at 4100 rpm at +4 $^\circ$C for 20 min and supernatant was used for H$_2$O$_2$ and MDA analyses. The MDA content was determined using spectrophotometry at 532 and 600 nm absorbance and defined as mmol kg fw$^{-1}$ with the formula [(Abs532 nm-Abs600 nm)/1.55 $\times$ 105] using the extinction coefficient of 155 mM$^{-1}$cm$^{-1}$ [42]. H$_2$O$_2$ was determined at 390 nm in a spectrophotometer, a standard graph created and defined as mmol kg fw$^{-1}$ [43].

The content of sucrose in the samples was determined in the spectrophotometer at a wavelength of 620 nm [44]. Proline extraction and proline content were determined according to method of Bates et al. [45]. The samples were measured at 520 nm with spectrophotometer and proline concentration was defined as mmol kg fw$^{-1}$. 
2.4.5. Catalase (CAT), Peroxidase (POD) and Superoxide Dismutase (SOD) Enzyme Activities

Fresh leaf samples were homogenized in the extraction solution according to the method specified by Angelini et al. [46] and Angelini and Federico [47] and the obtained supernatant was used to determine enzyme activities. CAT activity was determined by the decrease in absorbance of H$_2$O$_2$ at 240 nm [41]. POD activity of samples was determined at 436 nm and SOD activity at 560 nm with method of Liu et al. [41] by spectrophotometry.

2.4.6. Hormone Content

Extraction and purification processes were performed as described by Battal and Tileklioglu and Kuraishi et al. [48,49]. Methanol (80%) at $-40 \, ^\circ C$ was added to fresh samples, homogenized at 10 min (Ultra-Turrax, T-25, IKA GmbH & Co., Staufen im Breisgau, Germany) and then incubated for 24 h in dark. Afterwards, samples were dried at 35 $^\circ C$ and dissolved with 0.1 M KH$_2$PO$_4$ (pH 8.0). The hormones were determined by HPLC using a Zorbax Eclipse-AAA C-18 column (Agilent 1200 HPLC). Abscisic acid (ABA), cytokinin, gibberellic acid (GA), indole acetic acid (IAA), jasmonic acid, salicylic acid (SA) and zeatin were defined at 265 nm with a UV detector [50].

2.4.7. Mineral Element Content

Dry tomato leaves were ground and content of total N was determined by the Kjeldahl method using a Vapodest 10 Rapid Kjeldahl Distillation Unit (Gerhardt, Königswinter, Germany). For other mineral elements, analysis (P, K, Ca, Mg, S, Na, Zn, Fe, Mn, Cu, B and Cl) was performed using an inductively coupled plasma spectrophotometer (Optima 2100 DV, ICP/OES; PerkinElmer, Shelton, CT, USA) [51,52].

2.4.8. Statistical Analysis

Data were subject to analysis of variance (one-way ANOVA) and means comparison was made according to the Duncan multiple comparison tests using SPSS program [53].

3. Results

At the end of the experiment, we observed the negative effects of salinity on tomato seedlings and the ameliorative effects of H$_2$S were also very favorable (Figure 1).

3.1. Plant Growth Parameters

The effects of the treatments on plant height, stem diameter, the number of leaves, leaf area and the chlorophyll value in tomato seedlings were found to be statistically significant ($p < 0.001$). Plant height, stem diameter, the number of leaves, leaf area and the chlorophyll value (SPAD) of tomato seedlings decreased with increased salt stress. H$_2$S treatments alleviated the negative effect of salt on these parameters (Table 1). The plant heights were measured as 17 cm at 25 $\mu$M H$_2$S and 15 cm at 75 $\mu$M H$_2$S under 75 mM NaCl which were higher than the control treatments at the same salinity levels. We observed higher stem diameters in all H$_2$S treatments (25, 50, 75 and 100 $\mu$M H$_2$S) in both salt treatments (75 mM NaCl and 150 mM NaCl) as compared with control treatments under the same salinity levels.

The number of leaves under saline conditions was also increased with the 50 $\mu$M and 75 $\mu$M H$_2$S treatments (7.67 plant$^{-1}$ and 7.83 plant$^{-1}$) (Table 1). The highest leaf area was measured in 25 $\mu$M (464.85 cm$^2$ plant$^{-1}$) at 75 mM NaCl. Under severe salt stress (150 mM NaCl), the highest leaf areas (302.49 cm$^2$ plant$^{-1}$) were obtained from the 50 $\mu$M H$_2$S treatment. The chlorophyll SPAD values were higher in all H$_2$S treatments as compared with the control treatments under the same salinity levels (Table 1).
Figure 1. The effects of H\textsubscript{2}S and salt treatments on tomato seedlings.

Table 1. The effects of H\textsubscript{2}S treatments on plant height, stem diameter, number of leaves, leaf area and chlorophyll reading value in tomato seedlings under salt stress.

<table>
<thead>
<tr>
<th>Salt</th>
<th>Treatment</th>
<th>Plant Height</th>
<th>Stem Diameter</th>
<th>Leaf Number</th>
<th>Leaf Area</th>
<th>Chlorophyll Reading Value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>cm</td>
<td>Mm</td>
<td>Number plant\textsuperscript{−1}</td>
<td>cm\textsuperscript{2} plant\textsuperscript{−1}</td>
<td>SPAD</td>
</tr>
<tr>
<td>SI</td>
<td>T1</td>
<td>22.17\textsuperscript{b}</td>
<td>7.27\textsuperscript{ab}</td>
<td>9.67\textsuperscript{a}</td>
<td>784.91\textsuperscript{a}</td>
<td>52.80\textsuperscript{ab}</td>
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<td>III</td>
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<td>7.09\textsuperscript{ab}</td>
<td>9.00\textsuperscript{b}</td>
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<td>9.00\textsuperscript{b}</td>
<td>783.10\textsuperscript{a}</td>
<td>52.07\textsuperscript{ab}</td>
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<tr>
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<td>TIV</td>
<td>23.67\textsuperscript{ab}</td>
<td>7.31\textsuperscript{ab}</td>
<td>9.17\textsuperscript{b}</td>
<td>777.96\textsuperscript{a}</td>
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<td>7.32\textsuperscript{a}</td>
<td>8.83\textsuperscript{b}</td>
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<td>324.60\textsuperscript{c}</td>
<td>49.50\textsuperscript{c}</td>
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<td></td>
<td>TIV</td>
<td>16.33\textsuperscript{cd}</td>
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<td>7.83\textsuperscript{c}</td>
<td>325.05\textsuperscript{bc}</td>
<td>48.90\textsuperscript{c}</td>
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<tr>
<td></td>
<td>TV</td>
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<td>5.38\textsuperscript{d}</td>
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<td>50.97\textsuperscript{bc}</td>
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<td>SIII</td>
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<td>4.56\textsuperscript{g}</td>
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<td>4.93\textsuperscript{f}</td>
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<td>302.49\textsuperscript{cd}</td>
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<tr>
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<td>TIV</td>
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<td>6.17\textsuperscript{f}</td>
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<td>5.04\textsuperscript{ef}</td>
<td>7.02\textsuperscript{e}</td>
<td>253.46\textsuperscript{de}</td>
<td>45.33\textsuperscript{de}</td>
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</table>

SI: 0 mM NaCl (control), SII: 75 mM NaCl, SIII: 150 mM NaCl, T1: 0 \textmu M H\textsubscript{2}S, TII: 25 \textmu M H\textsubscript{2}S, TIII: 50 \textmu M H\textsubscript{2}S, TIV: 75 \textmu M H\textsubscript{2}S, TV: 100 \textmu M H\textsubscript{2}S. There is no statistical difference between the means shown with the same letter given in each column (p < 0.001).
The effect of the treatments on the fresh and dry weight of the plant and the fresh and dry weight of the roots in tomato seedlings were found to be statistically significant ($p < 0.001$). Plant fresh and dry weight and root fresh and dry weight decreased with increased salt stress. H$_2$S treatments alleviated the negative effect of salt on these parameters.

Plant fresh weight was higher in 25, 50 and 100 µM H$_2$S at 75 mM NaCl (13.40 g plant$^{-1}$, 12.81 g plant$^{-1}$ and 13.62 g plant$^{-1}$, respectively) as compared with the control treatment at the same salinity level (10.87 g plant$^{-1}$). Similarly, higher plant fresh weights were obtained from the application of 25, 50, 75 and 100 µM H$_2$S at 150 mM NaCl (7.81, 8.32, 7.65 and 8.65 g plant$^{-1}$) as compared with the control treatment at the same salinity level (6.04 g plant$^{-1}$). Root fresh weight was higher in all H$_2$S treatments than control treatments in each salinity level. Similarly, plant dry weight was higher in all H$_2$S treatments than the control in 75 mM and 150 mM NaCl. Root dry weight was high in all H$_2$S treatments under salt stress however the effect of H$_2$S treatments at 150 mM salt stress was not statistically significant (Table 2).

Table 2. The effects of H$_2$S treatments on plant fresh weight, root fresh weight, plant dry weight and root dry weight in tomato seedlings under salt stress.

<table>
<thead>
<tr>
<th>Salt</th>
<th>Treatment</th>
<th>Plant Fresh Weight</th>
<th>Root Fresh Weight</th>
<th>Plant Dry Weight</th>
<th>Root Dry Weight</th>
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<td>T1</td>
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<td>3.75$^{d}$</td>
<td>0.57$^{b}$</td>
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<td>T1</td>
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<td>0.84$^{h}$</td>
<td>0.16$^{d}$</td>
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<td>1.06$^{g}$</td>
<td>0.18$^{d}$</td>
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<td>TV</td>
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<td>2.30$^{e}$</td>
<td>1.14$^{g}$</td>
<td>0.18$^{d}$</td>
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</table>

SI: 0 mM NaCl (control), SII: 75 mM NaCl, SIII: 150 mM NaCl, T1: 0 µM H$_2$S, TII: 25 µM H$_2$S, TIII: 50 µM H$_2$S, TIV: 75 µM H$_2$S, TV: 100 µM H$_2$S. There is no statistical difference between the means shown with the same letter given in each column ($p < 0.001$).

3.2. LRWC, EL and Chlorophyll Content

Under saline water irrigation, while LRWC, chlorophyll a, chlorophyll b and total chlorophyll amounts in the tomato seedlings decreased, EL ratio increased. LRWC was higher with 25 and 50 µM H$_2$S treatments (60.55% and 60.09%) at 75 mM NaCl treatment, and with 25 µM H$_2$S (54.38%) at 150 mM NaCl compared with other treatments. The EL value, which increased with salinity, was higher in 0 µM H$_2$S (control) in 75 and 150 mM NaCl, while it was lower in both salt levels with H$_2$S (Figure 2).

Improved chlorophyll content was observed in all H$_2$S treatments under salinity. Chlorophyll a content was higher in 25 µM H$_2$S at 75 mM salt stress (5.62 mg g$^{-1}$) as compared with other treatments at the same salinity level. Similarly, chlorophyll b content was higher in 100 µM H$_2$S (4.30 mg g$^{-1}$) at 75 mM salt stress. Therefore, the total chlorophyll content in 25 and 100 µM H$_2$S (9.80 mg g$^{-1}$ and 9.81 mg g$^{-1}$) were higher at the same salinity level as compared with other treatments. Under severe salt stress, chlorophyll a content was higher in 100 µM H$_2$S treatment (5.28 mg g$^{-1}$) as compared with other H$_2$S treatments. Chlorophyll b content in 25 and 100 µM H$_2$S applications (2.84 mg g$^{-1}$ and 2.82 mg g$^{-1}$, respectively) was also higher as compared with other H$_2$S treatments at the
sustainability. The highest total chlorophyll content under 150 mM salt level was obtained from 100 µM H₂S (8.10 mg g⁻¹) (Figure 3).

![Graph](image)

**Figure 2.** The effects of H₂S treatments on LRWC and EL in tomato seedlings under salt stress. SI: 0 mM NaCl (control), SII: 75 mM NaCl, SIII: 150 mM NaCl, TI: 0 µM H₂S, TII: 25 µM H₂S, TIII: 50 µM H₂S, TIV: 75 µM H₂S, TV: 100 µM H₂S. There is no statistical difference between the means shown with the same letter given in each line (p < 0.001).

### 3.3. Photosynthetic Activity

In tomato seedlings, Pn, gs, Tr and Ci decreased due to salt concentrations in the irrigation water. The effect of treatments on plant Pn, gs, Tr and Ci content was found to be statistically significant (p < 0.001). Moreover, H₂S treatments alleviated the negative effect of salt on these parameters. The higher Pn were measured in 25 µM H₂S treatment (6.61 µmol m⁻²s⁻¹) at 75 mM NaCl, and in 75 and 100 µM H₂S treatments (4.34 µmol m⁻²s⁻¹ and 4.65 µmol m⁻²s⁻¹) in 150 mM NaCl as compared with other treatments in the same salinity levels. Under a mild salinity level (75 mM NaCl), gs was higher in 25, 50, 75 and 100 µM H₂S treatments as compared with the control treatment. Under severe salt stress (150 mM NaCl), higher gs values were obtained from 75 and 100 µM H₂S treatments as compared with other treatments under the same salt levels. Tr was higher in 25 and 100 µM H₂S treatments (2.90 mmol m⁻²s⁻¹ and 2.91 mmol m⁻²s⁻¹, respectively) in 75 mM NaCl. However, the highest Tr were obtained from 100 µM H₂S treatment (2.03 mmol m⁻²s⁻¹) in 150 mM NaCl as compared with other treatments under the same salt stress level. Higher Ci was measured from 50 and 75 µM H₂S applications (285.00 µmol mol⁻¹ and 282.62 µmol mol⁻¹, respectively) in 75 mM NaCl. Under severe salt stress (150 mM NaCl) all H₂S treatment doses improved Ci as compared with the control treatment (Figure 4).

### 3.4. H₂O₂, MDA, Sucrose and Proline Content

As salt concentrations increased in the tomato seedlings, H₂O₂, MDA, proline and sucrose contents also increased. The effects of treatments on plant H₂O₂, MDA, proline and sucrose content were found to be statistically significant (p < 0.001). While the content of H₂O₂ (9.70 mmol kg⁻¹), MDA (37.20 mmol kg⁻¹) and proline (0.10 mmol kg⁻¹) was lower in 100 µM H₂S treatment at 75 mM NaCl, the sucrose content (0.17%) was lower in 75 µM H₂S treatment at 150 mM NaCl (Figure 5).
Figure 3. The effects of H$_2$S treatments on chlorophyll-a, b and total chlorophyll in tomato seedlings under salt stress. SI: 0 mM NaCl (control), SII: 75 mM NaCl, SIII: 150 mM NaCl, TI: 0 µM H$_2$S, TII: 25 µM H$_2$S, TIII: 50 µM H$_2$S, TIV: 75 µM H$_2$S, TV: 100 µM H$_2$S. There is no statistical difference between the means shown with the same letter given in each bar ($p < 0.001$).
Figure 4. The effects of H₂S treatments on Pn, gs, Tr and Ci in tomato seedlings under salt stress. SI: 0 mM NaCl (control), SII: 75 mM NaCl, SIII: 150 mM NaCl, TI: 0 μM H₂S, TII: 25 μM H₂S, TIII: 50 μM H₂S, TIV: 75 μM H₂S, TV: 100 μM H₂S. There is no statistical difference between the means shown with the same letter given in each line (p < 0.001).

Figure 5. The effects of H₂S treatments on H₂O₂, MDA, proline and sucrose in tomato seedlings under salt stress. SI: 0 mM NaCl (control), SII: 75 mM NaCl, SIII: 150 mM NaCl, TI: 0 μM H₂S, TII: 25 μM H₂S, TIII: 50 μM H₂S, TIV: 75 μM H₂S, TV: 100 μM H₂S. There is no statistical difference between the means shown with the same letter given in each bar (p < 0.001).
3.5. CAT, SOD and POD Enzyme Activity

As salt concentrations increased in the tomato seedlings, CAT, POD and SOD enzyme activities increased. The effects of H$_2$S treatments on CAT, POD and SOD in the tomato seedlings were found to be statistically significant ($p < 0.001$). Lower CAT, POD and SOD activities were observed in 100 µM H$_2$S treatment compared with other treatments at both 75 mM NaCl and 150 mM NaCl salinity levels (Figure 6).

Figure 6. The effects of H$_2$S treatments on CAT, POD and SOD enzyme activity in tomato seedlings under salt stress. SI: 0 mM NaCl (control), SII: 75 mM NaCl, SIII: 150 mM NaCl, TI: 0 µM H$_2$S, TII: 25 µM H$_2$S, TIII: 50 µM H$_2$S, TIV: 75 µM H$_2$S, TV: 100 µM H$_2$S. There is no statistical difference between the means shown with the same letter given in each bar ($p < 0.001$).
3.6. Hormones and Mineral Contents

In the tomato seedlings, IAA, GA, SA, cytokinin, zeatin and jasmonic acid decreased under increased salt stress, while ABA increased. H$_2$S treatments alleviated the negative effect of salt on these parameters (Table 3).

<table>
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<th>Salt</th>
<th>Treatment</th>
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<th>GA</th>
<th>SA</th>
<th>Cytokinin</th>
<th>Zeatin</th>
<th>Jasmonic Acid</th>
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<td>7.16</td>
<td>35.01</td>
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</table>

The effects of salinity and H$_2$S treatments on the mineral content of tomato seedlings were found to be statistically significant. Plant N, P, K, Ca, Mg, S, Zn, Fe, Mn, Cu and B contents decreased with increased salinity. The lowest mineral contents were obtained from 150 mM NaCl. Under mild salt stress (75 mM NaCl), the highest N, P and K contents were measured from 25 µM H$_2$S treatment while the highest Ca contents were measured from 50 and 75 µM H$_2$S treatments, the highest Mg, Zn, Fe, Mn, Cu and B contents were obtained from 100 µM H$_2$S treatment, the highest S content was obtained from 75 µM H$_2$S treatment, the highest Zn, Fe, Mn, Cu and B contents were obtained in 100 µM H$_2$S treatment (Table 4).

<table>
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<tr>
<th>Salt</th>
<th>Treatment</th>
<th>N</th>
<th>P</th>
<th>K</th>
<th>Ca</th>
<th>Mg</th>
<th>S</th>
<th>Na</th>
<th>Zn</th>
<th>Fe</th>
<th>Mn</th>
<th>Cu</th>
<th>B</th>
<th>Cl</th>
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<tr>
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<td>1.18</td>
<td>0.32</td>
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<td>1.35</td>
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<td>1.37</td>
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<tr>
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</tbody>
</table>

SI: 0 mM NaCl (control), SII: 75 mM NaCl, SIII: 150 mM NaCl, T1: 0 µM H$_2$S, TII: 25 µM H$_2$S, TIII: 50 µM H$_2$S, TIV: 75 µM H$_2$S, TV: 100 µM H$_2$S. There is no statistical difference between the means shown with the same letter given in each column (p < 0.001).
Under severe salt stress (150 mM NaCl), the highest N and K contents were measured from 25 \( \mu M \) H\(_2\)S treatment, the highest P contents were obtained from 75 and 100 \( \mu M \) H\(_2\)S treatments, and the highest K content from 25 \( \mu M \) H\(_2\)S treatment. In addition, the highest Ca, Mg, S, Zn, Fe, Mn, Cu and B contents were obtained from 100 \( \mu M \) H\(_2\)S treatment. The Na and Cl contents of tomato seedlings increased with increased salt levels in the irrigation water, however, this increase was lesser in H\(_2\)S treatments. The least increases in Na and Cl content were observed in 100 \( \mu M \) H\(_2\)S treatment (Table 4).

4. Discussion

Salinity causes great economic losses in agricultural production because of yield and quality losses. The common opinion of the authorities is that a large part of arable agricultural land will become unusable in the future due to salinity. Therefore, in this study, the effects of H\(_2\)S treatment on tomato seedlings were investigated under salinity conditions and the results obtained are given in Table 1, Table 2, Table 3, Table 4, Figures 1–6.

In our study, the effect of severe salt stress (150 mM NaCl) was more detrimental on tomato seedlings when compared with mild salt stress levels (75 mM NaCl). A decrease was observed in the number of leaves and the leaf area at the highest concentration of salt (NaCl) in \textit{Vicia faba} [54]. However, this inhibitory effect of salt stress on plant height, stem diameter, leaf number and leaf area growth in tomato seedlings was lesser with H\(_2\)S treatments. Treatments of 25 and 75 \( \mu M \) H\(_2\)S on these parameters especially were more effective (Table 1). The curative effects of H\(_2\)S application on the plant damage caused by salt stress were stated in previous studies [33,55,56]. H\(_2\)S can have an ameliorative effect on the growth characteristics of plants under salt stress by mitigating the formation of ROS with the changes in antioxidant enzyme activity, the degradation effects of salinity on chlorophyll pigments and photosynthetic activity, and by modulating hormone and mineral element content.

In tomato seedlings, plant fresh and dry weight and root fresh and dry weight decreased significantly under salt stress (Table 2). It was announced earlier that salinity reduces plant growth in tomato, and salinity causes significant decreases in root fresh and dry weight and shoot fresh and dry weight [57]. Usually, salt stress causes a regression in the development of the above-ground part of the plant, related to defoliation partially mediated by ethylene production. However, a good root growth can provide nutrients and water uptake even in saline conditions and can increase plant tolerance to salinity [58]. In terms of plant fresh dry weight and root fresh dry weight, 25 \( \mu M \) H\(_2\)S and 100 \( \mu M \) H\(_2\)S treatments alleviated salt damage (Table 2). Similarly, Deng et al. [55] determined that the negative effect of salinity on the shoot and root fresh and dry weights of wheat seedlings was reduced by H\(_2\)S treatment, and seedling development was improved. On the other hand, Liu et al. [58] determined that low concentrations of H\(_2\)S regulated and supported better root growth than high concentrations.

Salt stress changes the lipid composition in the membrane and membrane structure, thus causing membrane damage [6]. In the tomato seedlings, LRWC decreased with increased salt levels, however, EL increased significantly (Figure 2). The increase in EL occurs due to damage in the cell membrane caused by salt stress. In plants, saline conditions cause water loss in the cell, disruption of the membrane plasma and the release of hydrolytic enzymes, which in turn causes disruption of the cytoplasm and a general slowdown in growth and a decrease in turgor [59]. However, we observed that H\(_2\)S treatments alleviated the decrease in LRWC under salt stress. The best application dose that increased LRWC under salt stress was 25 \( \mu M \) H\(_2\)S. The EL values that increased due to the increased salt stress were lower in 50 \( \mu M \) H\(_2\)S treatment. Mostofa et al. [60] reported that H\(_2\)S pretreatment reduces Na accumulation in rice plants under salt stress and thus reduces the water loss rate. The decrease in membrane permeability and LRWC was alleviated by the application of exogenous H\(_2\)S in salt-stressed maize and eggplant seedlings [17,33,61].

Chlorophyll content decreased significantly depending on the increased salt level, and the highest decrease was observed in plants grown under 150 mM NaCl (Figure 3).
Similarly, Shin et al. [62] also reported that chlorophyll a and chlorophyll b decreased in parallel with increased salt concentrations in tomatoes. In other studies, it has been stated that there is a significant decrease in chlorophyll a, chlorophyll b and total chlorophyll content together in beans with salinity [54], and that salt stress has a negative effect on the SPAD value in cucumber plants [63]. On the other hand, H$_2$S treatments applied to plants in a saline environment alleviated the reduction of salt stress in plants in terms of chlorophyll content. In particular, 100 µM H$_2$S treatment had positive effects on SPAD, chlorophyll a and total chlorophyll, and 25 µM H$_2$S had positive effects on chlorophyll b content at all salt levels (Table 1, Figure 3). Mostafa et al. [60] stated that H$_2$S improved the overall growth and biomass of rice plants under salt stress due to its role in protecting chlorophyll a, chlorophyll b, carotenoids and proteins from salt-induced damage.

One of the most important effects of stress conditions on plants is the decrease in photosynthetic activity. Salt stress causes a decrease in photosynthesis and ROS production [2]. $Pn$, $gs$, $Tr$ and $Ci$ values of the photosynthesis parameters we examined in the study decreased in parallel with the increased salt level. These decreases were 39–58%, 62–71%, 44–64% and 10–17%, respectively, in 75 mM NaCl and 150 mM NaCl conditions in tomato seedlings as compared with the control treatment (Figure 4). We observed that H$_2$S treatments increased photosynthetic properties with freshwater irrigation. In addition, the decrease in photosynthetic properties under salt stress was lower with H$_2$S treatments, and the negative effect of salt stress on these properties could be alleviated by exogenous H$_2$S treatments (Figure 4). Indeed, Chen et al. [26] stated that H$_2$S treatments in spinach increase photosynthetic enzyme expression and increase photosynthetic activity. In salt-stressed plants such as eggplant, rice and cucumber, an increase in photosynthetic activity was stated with exogenous H$_2$S treatments [17,18,33,60,64,65]. The increase in photosynthetic activity may be due to the positive effect on chlorophyll pigments caused by H$_2$S applications.

The increase in H$_2$O$_2$, MDA and proline contents was quite evident under salt stress, and the sucrose content increased slightly with salinity (Figure 5). Similarly, an increase in lipid peroxidation (MDA), H$_2$O$_2$ and sugar content were observed in lettuce exposed to salinity [14]. The reason for the increase in MDA and H$_2$O$_2$ in plants under salt stress is due to the increase of ROS and cell membrane damage with stress. While Li [66] found an increase in the content of proline and MDA in tomato seedlings with salinity in his study, there was also a change in the soluble sugar content depending on the increasing NaCl concentration. Shahba et al. [67] found that soluble sugars and the proline content in tomatoes increase with salinity.

Excessive H$_2$O$_2$ and MDA production occurred in tomato leaves exposed to salt stress, however, exogenous H$_2$S treatments significantly reduced MDA and H$_2$O$_2$ accumulation in tomato seedlings. According to the mean values, 100 µM H$_2$S treatment in tomato seedlings had a reducing negative impact of salinity on these parameters. The treatment of 100 µM H$_2$S in terms of H$_2$O$_2$, MDA and proline, and 75 µM H$_2$S in terms of sucrose were more effective doses for tomatoes grown under saline conditions (Figure 5). With these effects, it is evident that H$_2$S provides a protective effect against oxidative stress in tomato plants. H$_2$S provides a protective effect against oxidative damage by reducing H$_2$O$_2$ and MDA [19]. Similar results were announced earlier in maize, wheat and cucumber with exogenously applied H$_2$S [17,23,61]. Mostafa et al. [60], Wei et al. [68], Ekinci et al. [33] and Turan et al. [17] have stated earlier that the increase in MDA and H$_2$O$_2$ under salt stress can be decreased by exogenous H$_2$S treatments, and they underlined that the tolerance to plant salt stress can be increased with this application. Similarly, it was determined that H$_2$S treatments reduced the accumulation of proline and sucrose caused by salt stress in eggplant seedlings [33]. Furthermore, H$_2$S mitigated oxidative damage under salt stress by reducing the accumulation of H$_2$O$_2$ and MDA in tomato leaves in this study.

A complex antioxidant system has evolved to reduce and repair oxidative damage in plants. The activity of antioxidant enzymes such as CAT, SOD and POD is one of the effective ROS scavenging mechanisms [69]. In our study, antioxidant enzyme activity increased in tomato seedlings to reduce oxidative damage caused by salinity (Figure 6).
However, we observed that H$_2$S treatments reduced this effect of salinity on enzyme activity. There were significant decreases in CAT, POD and SOD activities under salt stress in tomato seedlings, especially with the treatment of 100 µM H$_2$S (Figure 6). On the other hand, it was determined earlier that H$_2$S treatment could mitigate oxidative stress by improving the expression and activities of antioxidant enzymes [56]. Furthermore, Sun [70] found that H$_2$S application increased SOD and POD activities in cucumber. This situation may be due to differences between species/generotypes and environmental conditions.

It was also determined that H$_2$S treatment significantly reduced H$_2$O$_2$ accumulation and lipid peroxidation [20]. H$_2$S helps antioxidant defense systems by interacting with other molecules such as nitric oxide (NO), ROS and phytohormones, which alleviate the harmful effects of abiotic stresses [71]. It has been determined that both the endogenous and exogenous treatment of H$_2$S play an important role in promoting salt tolerance by reducing ROS accumulation in eggplant and tomato [21].

Phytohormones in plants control the plant’s reactions to salinity [72]. In this study, important changes occurred in plant hormone content with different salt levels. ABA, which is one symptom of stress, increased significantly due to the increased salt stress (Table 3). It is known that ABA has an important role in the stress response and/or adaptation. Exposure of the plant to salinity causes an increase in the ABA level, which is generally related to leaf or soil water potential [72]. However, in our experiment, the increase in ABA content caused by salinity decreased with 75 µM H$_2$S treatment in tomato seedlings under salt stress (Table 3). H$_2$S interacts with important phytohormones while regulating plant responses to abiotic stresses [73]. It has been determined that exogenous H$_2$S administration can participate in ABA-induced stomatal closure in stressed plants [74], and that it positively modulates ABA signaling in guard cells through persulfidation proteins encoded by OST1 and SnRK2.6 genes [75].

The interactions of H$_2$S with hormones such as IAA and GA regulate plant growth and development under stress conditions. Indeed, under stress conditions, it has been reported that the H$_2$S-phytohormone interaction enhances the tolerance to some abiotic stress conditions in plants. In addition, it has been reported that H$_2$S, together with SA and JA, increases the tolerance of the plant under normal and stress conditions by regulating metabolic activities such as stonal closure, maturation and senescence [76]. H$_2$S interacts with IAA in the development of lateral roots [77], and in tomato seedlings, stimulate up-regulation of an auxin-dependent gene [78].

In our study, there was a significant decrease in plant mineral content except for Na and Cl (Table 4). The exogenous application of H$_2$S improved the mineral substance content in tomato seedlings under salt stress (Table 4). H$_2$S is effective in the nutrient balance in plants and on the survival of the plant under abiotic stress [71]. Mostofa et al. [60] reported that NaHS increases the amount of essential minerals required to activate the crucial processes in salt stress adaptation. It has been stated that H$_2$S interacts with Ca and participates in the regulation of antioxidant defense and ion homeostasis in salt stress [79]. The findings obtained from this study show that H$_2$S can help to mitigate the decrease in mineral matter content under salt stress.

When evaluated in general, the effects of H$_2$S on salt stress damage in tomato seedlings were ameliorative on plant morphological, physiological and biochemical properties in our study. The effect of H$_2$S in mitigating salt stress damage in tomato seedlings might be due to increased photosynthetic activity, changed antioxidant enzyme activity and coordinated signal transduction pathways related to stress response [23,80]. In addition, it is stated that the positive effects of H$_2$S on plant physiology and biochemistry may be due to the protection of the cellular structure, which is compromised by salinity, with ROS homeostasis, membrane integrity, osmotic balance and maintenance of K$^+$/Na$^+$ [80]. In this study, a similar response of tomato seedlings to salt stress was observed in terms of some of the parameters that were examined with these metabolisms of H$_2$S.
5. Conclusions

In this study, the effects of H$_2$S applications with different doses under different salt stress levels on tomato seedlings were investigated and the results were examined in regard to various morphological, physiological and biochemical aspects. It was found that there was a significant decrease in plant growth and development in parallel with the increased salt stress. The exogenous application of H$_2$S on the leaves improved tomato seedlings’ tolerance to salt stress. The effect of 25 µM H$_2$S treatment on plant growth parameters and 75 and 100 µM H$_2$S treatments in terms of physiological and biochemical properties were more pronounced in tomato. In tomato seedlings under salt stress, H$_2$S has a protective role against the negative effects of stress with its effects such as protecting membrane permeability, increasing photosynthetic activity, changes in antioxidant enzyme activity, modulating the hormone level and mineral content in line with the plant’s needs against stress.

As a result, based on the curative effects of H$_2$S in tomato seedlings grown under salt stress, it can be considered as an alternative treatment for cultivation in problematic areas with low irrigation water quality. Moreover, it will be useful to investigate the effects at the molecular level at a later stage for more detailed results. In addition, as a continuation of the study, experiments that take place until fruit yield and ripening in plants can be conducted to optimize the application doses of H$_2$S.

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