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Effect of exogenously applied salicylic acid on cadmium chloride-induced oxidative stress and nitrogen metabolism in tomato (*Lycopersicon esculentum* L.)

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Abstract: Salicylic acid (SA) has a key role in the formation of induced systemic resistance and hypersensitive cells, but it has also been indicated to be effective in the formation of strong responses against abiotic stresses such as drought, cold, heavy metal toxicity, and osmotic stress. This study has demonstrated a connection between the total soluble protein and the nitrate reductase (NR) activity. In 20 µM $CdCl_2$, 40 µM $CdCl_2$ + 0.5 mM SA, and 100 µM $CdCl_2$ + 0.5 mM SA treatments, on the 1st day following the treatment an increase was detected in the NR activity when compared to the control group ($P < 0.05$); however, there was a decrease in the NR activity on the 3rd and 5th days of the treatment. An increase in protein amount was detected on the 1st day following the treatment whereas the amount of total soluble protein decreased on the 5th day in comparison to the control group. All concentrations of $CdCl_2$ + 0.5 mM SA treatments caused an increase in the amounts of malondialdehyde, H_2O_2, and NO_3^- on the 5th day of the treatment.

Key words: Cadmium, H_2O_2, nitrate, nitrate reductase activity, malondialdehyde, salicylic acid, tomato

1. Introduction

Heavy metals are released from many sources such as industrial activities, municipal waste, mining operations, fertilizer and pesticide use in agriculture, exhaust gases of motor vehicles, and volcanic activities. These sources constitute major problems in agriculture, husbandry, and forestry. Heavy metals (Cd, Zn, Cr, Pb, etc.) lead to the enrichment of the soil in terms of toxic metals. Accumulation of these metals in the soil and the environment poses a gradually increasing danger for almost all kinds of organisms, including plants and human beings (1,2).

Cadmium is one of the most destructive heavy metals that cause oxidative stress in plants (3). Found in the air, soil, and water, Cd is a strong environmental pollutant and has toxic effects on plants, animals, and humans. Due to its neurotoxic, mutagenic, and carcinogenic effects as well as its high solubility in water, cadmium can easily enter the human body through the food chain (4). This metal can form a complex with side groups of many organic molecules such as proteins and it interacts with several cellular metabolisms (such as lipid and protein metabolism, respiration, photosynthesis, and nitrate reductase [NR] activity). In addition, it damages biomolecules including membrane lipids and leads to the formation of reactive oxygen species (ROS) such as hydrogen peroxide (H_2O_2), which causes oxidative stress (5).

Salicylic acid (SA) is a signaling molecule in plants that is required for stimulating specific responses against various biotic and abiotic stresses. Many researchers have suggested that this molecule may be a new plant regulator (6). Recent studies suggested that SA has a protective effect against oxidative damage induced as a result of heavy metal pollution. SA is a molecule that is directly required in the antioxidant system (7). SA was discovered to be a signaling molecule necessary for the local and systemic resistance of plants after a pathogen attack (8). Following an infection, the SA level increases and leads to the synthesis of pathogenesis-related proteins as well as the induction of hypersensitive systemic acquired resistance. However, molecular events included in SA signaling are not yet fully understood; therefore, the studies in this area are increasing.

The aim of this study was to investigate biochemical parameters such as NR activity, protein, nitrate (NO_3^-), H_2O_2, and lipid peroxidation product malondialdehyde (MDA) in tomato seedlings exposed to $CdCl_2$ and $CdCl_2$ + SA treatments on certain days (1st, 3rd, and 5th days).

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2. Materials and methods

2.1. Plant material and growth conditions

Tomato seeds were left in 0.75% sodium hypochlorite for 1–2 min and then washed with sterile water and soaked in beakers filled with 700 mL/L ethanol for 5 min; afterwards, they were washed thoroughly with sterile water including 1–2 drops of Tween 20 per liter. Washed seeds were placed on glass petri dishes of 9 cm in diameter, each soaked with 5 mL of sterile water. Seeds were left for 3 days at 25 °C for swelling up. Later, 3 of the seeds left for swelling up were sown inside holes with 5-cm spacing in pots of 15 × 75 × 12 cm, which were filled with sifted garden soil, sifted composted manure, and fine sand (1:1:1). When seedlings left for germination and watered every other day reached the 2–3 leaf phase, those with equal growth were selected from the available seedlings and rarefaction of pots was undertaken so that there was only 1 seedling in each hole; when the seedlings reached the 6–7 leaf phase, they were harvested.

The roots of the tomato seedlings were washed with tap water and disinfected by being kept in 0.75% sodium hypochlorite for 1–2 min. They were then washed with sterile distilled water with 1–2 drops of Tween 20 per liter. The seedlings were placed into sterile glass bottles full of Hoagland nutrient solution with 20, 40, 80, 100, and 200 μM $CdCl_2$ and 20 μM $CdCl_2$ + 0.5 mM SA, 40 μM $CdCl_2$ + 0.5 mM SA, 80 μM $CdCl_2$ + 0.5 mM SA, 100 μM $CdCl_2$ + 0.5 mM SA, and 200 μM $CdCl_2$ + 0.5 mM SA. Full Hoagland nutrient solution was used as the control group in our experiments. A total of 30 seedlings were put into sterile glass bottles containing 400 mL of Hoagland solution. For each treatment, 3 glass bottles were prepared. According to the randomized block design model, random tomato seedlings were taken on the 1st, 3rd, and 5th days; the leaves were separated, frozen in liquid nitrogen, and stored at –80 °C until analysis.

2.2. Total protein assay

Total soluble protein content was determined according to the method described by Kurkela et al. (9). This homogenization was conducted with a chilled mortar and pestle using a buffer containing cold 50 mM Tris-HCl (pH 6.8), 1% (v/v) 2-β mercaptoethanol, and 50 mg/L PMSF. The homogenate was centrifuged at 15,800 × *g* at 4 °C for 5 min. Supernatant was stored at –20 °C for analysis. Protein concentrations were measured (595 nm) using a modified procedure with BSA as the standard protein according to the method described by Bradford (10).

2.3. NR (EC 1.6.6.1) assay

NR activity was assayed by the method of Jaworski (11). Plant leaves were incubated in 5 mL of incubation medium. The incubation medium was prepared by mixing 0.1 N KNO_3, 0.1 M phosphate buffer of pH 7.5, 0.1% Triton X-100, and 1% propanol. Incubation was carried out in the dark for 1 h at 28 ± 2 °C. Aliquots of 0.2 mL from the incubation mixture were analyzed for nitrite after 60 min. To the incubation medium, distilled water, 3% sulfanilamide in 3 N HCl, and 0.02% N-(l-naphthyl) ethylenediamine dihydrochloride were added in quick succession. This was incubated for 15 min in darkness for color development and absorbance was read at 540 nm with a spectrophotometer. The amount of nitrite (NO_2^-) formed was expressed as nanomoles of NO_2^- produced per minute per milligram fresh weight using a sodium nitrite ($NaNO_2$) standard curve.

2.4. Determination of NO_3^- content

NO_3^- content was assayed by the method of Ruiz et al. (12). Plant leaves were ground at 4 °C in 50 mM potassium phosphate buffer (pH 7.5) containing 2 mM EDTA, 1.5% (w/v) soluble casein, 2 mM DTT, and 1% (w/v) insoluble PVP. The homogenate was centrifuged at 3000 × *g* for 5 min, and then the supernatant was centrifuged at 20,000 × *g* for 20 min.

NO_3^- content in the plant leaves was measured by the SA method (13). The aliquot of extract was mixed thoroughly with 5% (w/v) SA in concentrated H_2SO_4. After 20 min at room temperature, 2 M NaOH was added slowly to raise the pH above 12. The samples were cooled to room temperature, and absorbance at 410 nm was determined by spectrophotometer.

2.5. Determination of H_2O_2 content

Hydrogen peroxide content in the leaves of the tomatoes was determined in accordance with the method of Velikova et al. (14). Samples were homogenized in an ice bath with 0.1% (w/v) trichloroacetic acid (TCA). The homogenate was centrifuged at 12,000 × *g* for 15 min and then 0.5 mL of the supernatant was added to 0.5 mL of 10 mM potassium phosphate buffer (pH 7.0) and 1 M KI. The absorbance of the supernatant was measured at 390 nm. The content of H_2O_2 was calculated by comparison with a standard calibration curve by using different concentrations of H_2O_2.

2.6. Determination of MDA content

The level of lipidperoxidation in the tomato leaves was estimated by the amount of MDA. The MDA concentration in tomato leaves was determined by the thiobarbituric acid (TBA) reaction in accordance with the method of Heath and Packer (15). Plant tissue was homogenized in 0.1% (w/v) TCA. The homogenate was centrifuged at 10,000 × *g* for 5 min and then 1.5 mL of 20% TCA containing 0.5% (w/v) TBA was added to the supernatant. The mixture was then centrifuged at 5000 × *g* and the absorbance was measured at 532 nm. The optical density for nonspecific absorption at 600 nm was subtracted from the actual measurement at 532 nm. The concentration of MDA was calculated using an extinction coefficient of 155 mM^{-1} cm^{-1}.

2.7. Statistical analysis

The trial was organized to create an experimental design with 3 repetitions in randomized blocks. Samples taken from the leaves of the control, Cd treatment, and Cd + SA treatment tomato seedlings with 6–7 leaves were also analyzed by applying factorial variance analysis techniques to the data obtained as a result of the analyses (in terms of protein, NR, nitrate, H_2O_2, and MDA), with 3 repetitions (n = 3). Data presented are mean values ± standard error of measurement (SEM) for 3 replicates. Data sets were analyzed with 2-way ANOVA (the factors were days and treatments) and means compared with the Student–Newman–Keuls multiple range test. Variance analysis was conducted using SPSS 15. The alpha level was set at 5%.

3. Results

In our study, tomato plant was exposed to 20, 40, 80, 100, and 200 μM $CdCl_2$ and 20 μM $CdCl_2$ + 0.5 mM SA, 40 μM $CdCl_2$ + 0.5 mM SA, 80 μM $CdCl_2$ + 0.5 mM SA, 100 μM $CdCl_2$ + 0.5 mM SA, and 200 μM $CdCl_2$ + 0.5 mM SA treatments. Excluding the 80 and 200 μM $CdCl_2$ treatments on the 1st day and the 20 μM $CdCl_2$ treatment on the 3rd day following the initial treatment, increases in protein amounts were detected in the leaves of $CdCl_2$-treated tomato seedlings. When compared with the control group, the most significant increases were found in the 100 μM $CdCl_2$ treatment on the 1st day and the 200 μM $CdCl_2$ treatment on the 3rd day ($P < 0.05$). On the 5th day following the treatment, a decrease in protein amount was detected in parallel with the increase in $CdCl_2$ concentration ($P < 0.05$) (Figure 1).

When $CdCl_2$ and SA were co-administered, SA was found to increase the protein amount on the 1st day following the treatment in all concentrations ($P < 0.05$). When compared with the control group, the highest increase was observed on the 3rd day in the 20 μM $CdCl_2$ + 0.5 mM SA treatment with an increase of 46.83% ($P < 0.05$) (Figure 1). In other words, 0.5 mM SA was found to inhibit to a large extent the toxicity of individually administered 20 μM $CdCl_2$. When compared with the control group, on the 5th day following the treatment, 4.33% reduction was observed in the amount of protein formed as a result of 20 μM $CdCl_2$ + 0.5 mM SA treatment; however, this difference was not statistically significant. On the other hand, excluding the 100 μM $CdCl_2$ + 0.5 mM SA treatment, in other treatments of SA + $CdCl_2$, a decrease in protein amount was observed in parallel with the increase in $CdCl_2$ concentration ($P < 0.05$) (Figure 1).

A decrease in NR activity was observed in the leaves of $CdCl_2$-treated tomato seedlings in all concentrations except for the 20 μM $CdCl_2$ treatment on the 1st day following the treatment ($P < 0.05$) (Figure 2). In the 20 μM $CdCl_2$ treatment, an increase of approximately 36.46% was detected in the NR activity when compared to the control group ($P < 0.05$). Enzyme activity decreased in all concentrations in comparison with the control group on the 3rd and 5th days following the treatment ($P < 0.05$) (Figure 2).

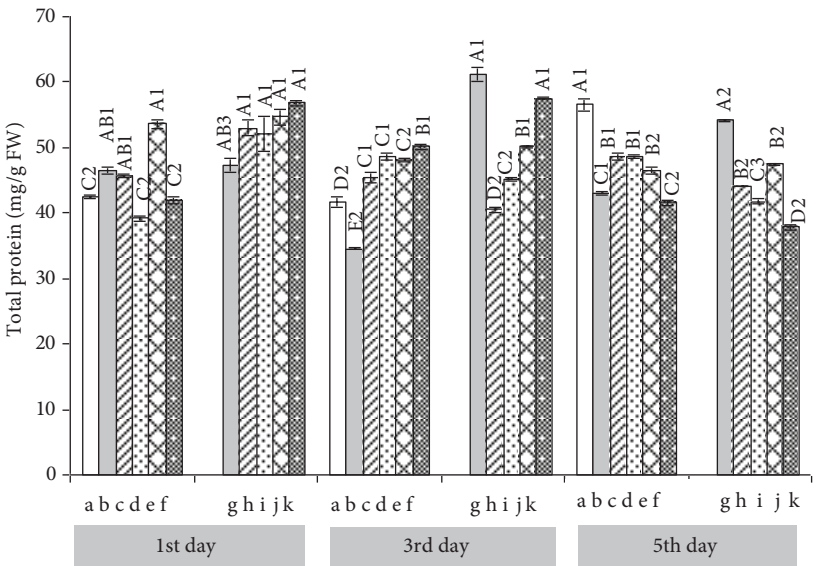

Figure 1. Changes in the total protein content in the leaves of tomato plants treated with cadmium and cadmium + salicylic acid (**a:** control, **b:** 20 μM $CdCl_2$, **c:** 40 μM $CdCl_2$, **d:** 80 μM $CdCl_2$, **e:** 100 μM $CdCl_2$, **f:** 200 μM $CdCl_2$, **g:** 20 μM $CdCl_2$ + 0.5 mM SA, **h:** 40 μM $CdCl_2$ + 0.5 mM SA, **i:** 80 μM $CdCl_2$ + 0.5 mM SA, **j:** 100 μM $CdCl_2$ + 0.5 mM SA, **k:** 200 μM $CdCl_2$ + 0.5 mM SA). The statistical significance is indicated above the bars, with capital letters representing concentration differences in the same day and numbers representing differences in days at the same concentration.

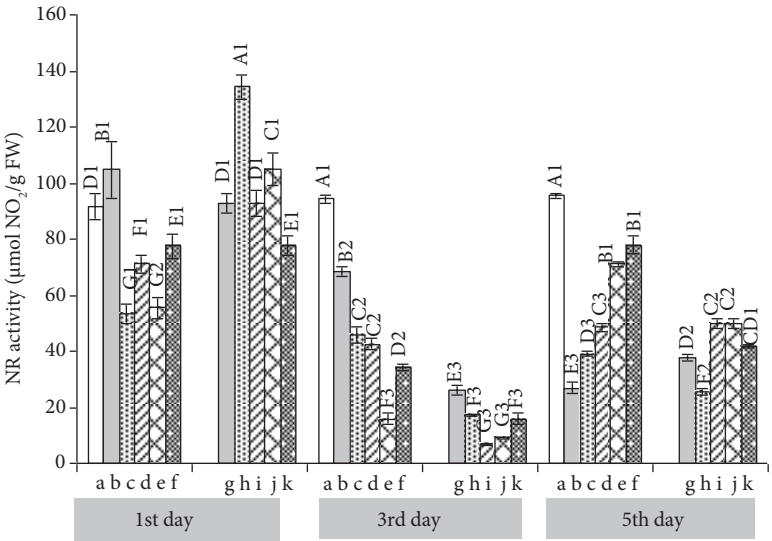

Figure 2. Changes in nitrate reductase activity in the leaves of tomato plants treated with cadmium and cadmium + salicylic acid (**a:** control, **b:** 20 µM $CdCl_2$, **c:** 40 µM $CdCl_2$, **d:** 80 µM $CdCl_2$, **e:** 100 µM $CdCl_2$, **f:** 200 µM $CdCl_2$, **g:** 20 µM $CdCl_2$ + 0.5 mM SA, **h:** 40 µM $CdCl_2$ + 0.5 mM SA, **i:** 80 µM $CdCl_2$ + 0.5 mM SA, **j:** 100 µM $CdCl_2$ + 0.5 mM SA, **k:** 200 µM $CdCl_2$ + 0.5 mM SA). The statistical significance is indicated above the bars, with capital letters representing concentration differences in the same day and numbers representing differences in days at the same concentration.

When $CdCl_2$ and SA were co-administered, generally an increase in NR activity was detected on the 1st day following the treatment in comparison with the control group; however, only the increases in the 40 µM $CdCl_2$ + 0.5 mM SA and 100 µM $CdCl_2$ + 0.5 mM SA treatments were found to be statistically significantly higher than the other $CdCl_2$ + SA treatments ($P < 0.05$). On the 3rd day following treatment, a decrease was observed in the NR activity in comparison to the control group ($P < 0.05$) (Figure 2).

When compared to the control group, the amount of NO_3 in the leaves of 40 µM $CdCl_2$-treated tomato seedlings decreased on the 1st day following the treatment. On the 5th day, an increase was observed only in the 40 and 80 µM $CdCl_2$ treatments (Figure 3).

When $CdCl_2$ and SA were co-administered, the amount of NO_3 increased in all days and in all concentrations ($P < 0.05$) (Figure 3).

The amount of H_2O_2 in the leaves of $CdCl_2$-treated tomato seedlings increased on the 1st day after treatment in the 40 and 80 µM $CdCl_2$ treatments and on the 3rd day after treatment in 40 µM $CdCl_2$ treatments. In the 20 µM and 80 µM $CdCl_2$ treatments, an increase was detected in H_2O_2 content when compared to the control group ($P < 0.05$). At high $CdCl_2$ concentrations (80, 100, and 200 µM $CdCl_2$) the amount of H_2O_2 significantly decreased in comparison to the control ($P < 0.05$) (Figure 4).

When $CdCl_2$ and SA were co-administered, in contrast to the 1st day following the treatment, the amount of H_2O_2 increased in all concentrations on the 5th day ($P < 0.05$) (Figure 4).

When compared to the control group, an increase in the amount of MDA was observed in the leaves of $CdCl_2$-treated tomato seedlings in all concentrations except the 20 and 40 µM Cd treatments on the 1st day following the treatment ($P < 0.05$) (Figure 5). When compared to the control group, an increase in the amount of MDA was observed in all concentrations on the 3rd and 5th days following the treatment ($P < 0.05$) (Figure 5).

When $CdCl_2$ and SA were co-administered, in comparison to the control, the amount of MDA increased in low concentrations (20 µM $CdCl_2$ + 0.5 mM SA, 40 µM $CdCl_2$ + 0.5 mM SA, 80 µM $CdCl_2$ + 0.5 mM SA) on the 1st day following the treatment and in high concentrations (80 µM $CdCl_2$ + 0.5 mM SA, 100 µM $CdCl_2$ + 0.5 mM SA, 200 µM $CdCl_2$ + 0.5 mM SA) on the 5th day following the treatment ($P < 0.05$) (Figure 5).

4. Discussion

Toxicity induced by Cd causes the accumulation of a strong oxidant, H_2O_2; when the antioxidant system is insufficient, it leads to oxidative damage (i.e. MDA), membrane leakage, and ultimately cell death (16). Many physiological events including the nitrogen and the carbohydrate metabolisms are influenced by the presence of cadmium (17). Some studies showed that the enzymes of N metabolism are affected by the stress induced by cadmium (18). Mosulén et al. (19) reported that high concentrations of cadmium in the growth environment inhibited the first factor in the organization of NR induction, the NO_3^- uptake. Srivastava et al. (20) reported that total protein content and NR

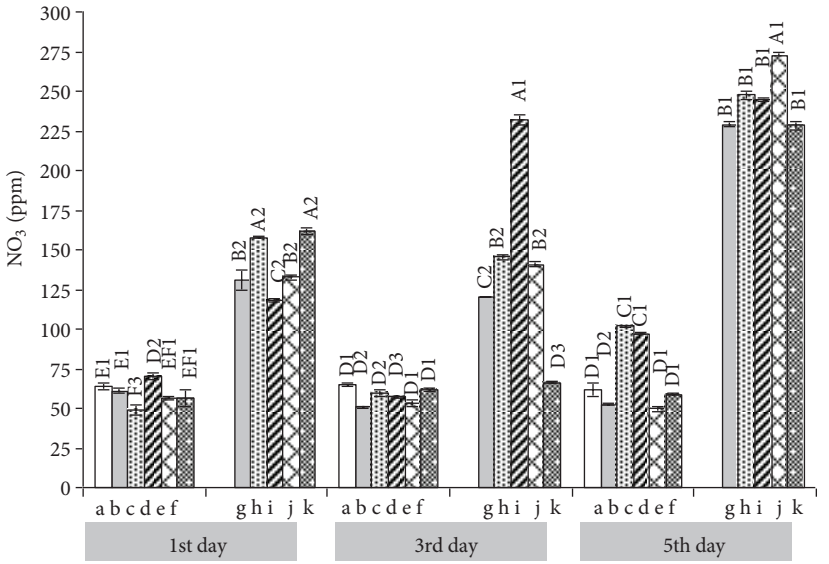

Figure 3. Changes in the nitrate content in the leaves of tomato plants treated with cadmium and cadmium + salicylic acid (**a:** control, **b:** 20 μM $CdCl_2$, **c:** 40 μM $CdCl_2$, **d:** 80 μM $CdCl_2$, **e:** 100 μM $CdCl_2$, **f:** 200 μM $CdCl_2$, **g:** 20 μM $CdCl_2$ + 0.5 mM SA, **h:** 40 μM $CdCl_2$ + 0.5 mM SA, **i:** 80 μM $CdCl_2$ + 0.5 mM SA, **j:** 100 μM $CdCl_2$ + 0.5 mM SA, **k:** 200 μM $CdCl_2$ + 0.5 mM SA). The statistical significance is indicated above the bars, with capital letters representing concentration differences in the same day and numbers representing differences in days at the same concentration.

activity were reduced due to various Cd treatments. This metal caused significant decreases in NR activity in our study, as well, and this in turn led to a decrease in the nitrate assimilation of plants. Debouba et al. (21) found an increase in NR activity in the leaves of tomato seedlings exposed to 100 mM NaCl stress and a decrease in the amount of NO_3 depending on the increase in the duration of treatment. Dinakar et al. (22) found a decrease in NR activity in *Arachis hypogaea* seedlings exposed to 100 μM Cd stress. In 100 μM $CdCl_2$ treatment and the other treatments, NR activity in the leaves of tomato seedlings was observed to generally decrease in comparison to the control groups (days 3 and 5) in our study. In other study, changes were detected in NR activity levels depending on the time after treatment. When the 25, 50, and 100 μM Cd treatments were compared in terms of NR activity in *Cicer arietinum* L., it was observed that the amounts of NR at 30 days were significantly higher than 60 days (23). In our study, when compared to control groups, the maximum increase in NR activity was observed in the leaves of tomato seedlings on day 1 following treatment.

The important role of SA is considered to be an inherent regulator of plant metabolism. Previous studies have shown that exogenous SA treatment caused different biochemical responses depending on the SA concentration and the duration of treatment (24). There are many studies on this subject. Short-term treatments of SA were found to have more positive results on the plant growth, photosynthesis, and antioxidant system (25). Exogenously administered 0.01, 0.1, and 0.5 mM SA had a stimulatory effect on the maize plant, whereas high concentrations such as 1, 5, and 10 mM SA were determined to have an inhibitory effect and these concentrations led to a reduction in the NR activity. These concentrations of SA play an active role in the regulation of NR activity (26). Total protein content increased in a study on SA-treated soybean and this increase was suggested to be related to the increase in NR activity (27). In another study on maize plant, low concentrations (10^{-5} M) of SA were detected to cause an increase in NR activity (28). However, high concentrations (10^{-3} to 10^{-4} M) were proven to have an inhibitory effect. In the *Cicer arietinum* L. plant, SA increased nitrogen uptake and NR activity at low concentrations (10^{-5} M), but a decrease was detected in NR activity when compared to 10^{-5} M at high concentrations (10^{-4} M) (29). Canakci (30) analyzed the changes in biochemical mechanisms in pepper as a result of 0.3, 1.5, 5, and 10 mM SA treatments. The 0.3 mM SA treatment caused some changes, but these changes were not statistically significant. The 5 and 10 mM SA treatments demonstrated an inhibitory effect. Inhibitory high SA concentrations were more dominant than stimulatory low SA concentrations. He determined that the administered SA concentrations had a 2-way effect on the plants.

Salicylates are molecules effective on the protein synthesis mechanism, which is defined as the control mechanism. The decrease in the amount of protein is due to the inhibition of NR activity (31). On the other hand,

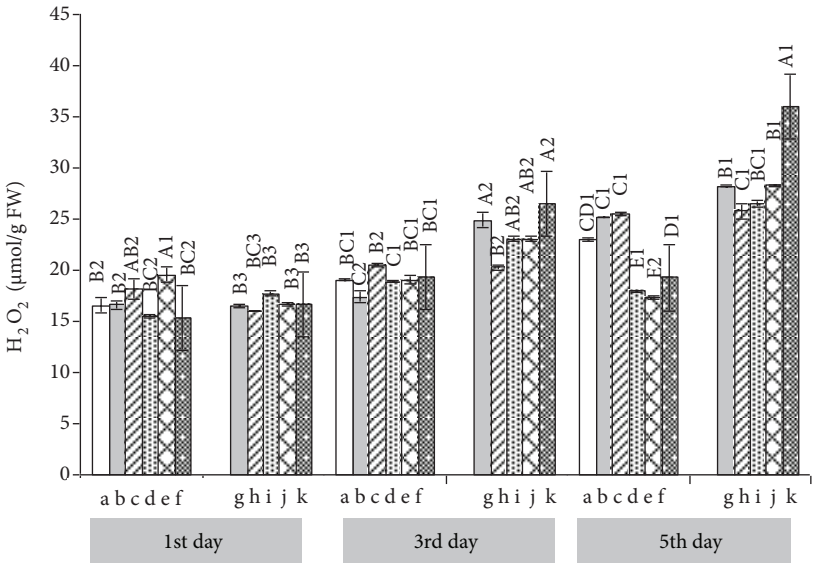

Figure 4. Changes in the hydrogen peroxide content in the leaves of tomato plants treated with cadmium and cadmium + salicylic acid (**a:** control, **b:** 20 µM $CdCl_2$, **c:** 40 µM $CdCl_2$, **d:** 80 µM $CdCl_2$, **e:** 100 µM $CdCl_2$, **f:** 200 µM $CdCl_2$, **g:** 20 µM $CdCl_2$ + 0.5 mM SA, **h:** 40 µM $CdCl_2$ + 0.5 mM SA, **i:** 80 µM $CdCl_2$ + 0.5 mM SA, **j:** 100 µM $CdCl_2$ + 0.5 mM SA, **k:** 200 µM $CdCl_2$ + 0.5 mM SA). The statistical significance is indicated above the bars, with capital letters representing concentration differences in the same day and numbers representing differences in days at the same concentration.

inhibition of the NR activity blocks nitrate reduction. Fariduddin et al. (26) reported that low concentrations of SA increased NR activity whereas high concentrations inhibited it. In our study, excluding the 1st day, the beneficial role of SA on plants exposed to Cd appeared not to be related to the activation of NR. The 0.5 mM SA application decreased the toxic effect of cadmium on the 1st day; however, it accelerated the effect of cadmium damage on the 3rd day of treatment. That is to say, 0.5 mM SA treatment demonstrated an inhibitory effect. The NR activity significantly decreased on the 3rd and 5th days of the treatment in comparison to the control; however, the amount of NO_3 increased with the increasing duration of treatment (especially on the 5th day). This increase may be due to the blocking of NO_3 reduction depending on the loss of NR activity. In $CdCl_2$ treatments, the decrease in NR activity also caused a decrease in the amount of protein on the 5th day of the treatment. The decrease in NR activity also may have caused a decrease in the amount of protein. This reduction in nitrogen compounds can be explained by the limited nitrogen assimilation.

In plants, stress factors increase ROS such as H_2O_2. This increase in ROS in plants causes oxidative damage to biomolecules such as lipids, proteins, and nucleic acids. SA was determined to stimulate the antioxidant system in the tomato plant under drought stress (32), in *Brassica juncea* under salinity stress (33), and in *Oryza sativa* exposed to cadmium stress (34) because SA has an affinity against some antioxidants such as catalase. Nevertheless, contrary to these observations, a decrease in the activity of antioxidant system was also reported for SA-treated rice (35).

Free radicals such as H_2O_2 are harmful products of many normal metabolic events. To prevent the damage of this product, H_2O_2 should be swiftly converted into another less harmful product (36). Certain concentrations of SA were determined to cause inhibition of antioxidant enzymes catabolizing H_2O_2. The inhibition increases the accumulation of H_2O_2 (37); consequently, this causes H_2O_2 to have a key role in the generation of defense responses in the plant. However, this mechanism is not generalizable. In a study on *Arabidopsis* leaves, it was observed that increasing SA concentration increased H_2O_2 level, lipid peroxidation, and the amount of oxidized proteins, hence increasing the activities of antioxidant enzymes (38). Under these circumstances, SA is a prooxidant and phytotoxin. In several studies, SA was reported to act like a versatile ROS under some abiotic stress conditions such as UV, temperature, and salinity. Senaratna et al. (39) reported it to act like a molecule involved in the formation of positive defense responses. Zhang et al. (40) said that seed-soaking pretreatment with 100 µM SA decreased 50 µM Cd-induced production of H_2O_2 in *Phaseolus aureus* and *Vicia sativa* roots on the 1st day following the treatment and SA induced Cd tolerances in *P. aureus* and *V. sativa*. In accordance with our results, 0.5 mM SA solution for 24 h alleviated negative effects on 40 µM $CdCl_2$-treated tomato leaves, but 0.5 mM SA + 200 µM $CdCl_2$ treatment

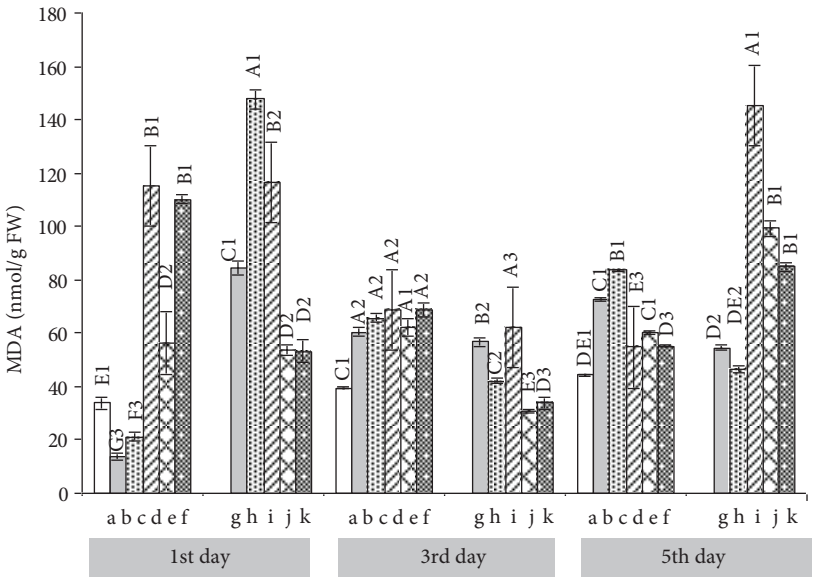

Figure 5. Changes in the MDA amount in the leaves of tomato plants treated with cadmium and cadmium + salicylic acid (**a:** control, **b:** 20 μM $CdCl_2$, **c:** 40 μM $CdCl_2$, **d:** 80 μM $CdCl_2$, **e:** 100 μM $CdCl_2$, **f:** 200 μM $CdCl_2$, **g:** 20 μM $CdCl_2$ + 0.5 mM SA, **h:** 40 μM $CdCl_2$ + 0.5 mM SA, **i:** 80 μM $CdCl_2$ + 0.5 mM SA, **j:** 100 μM $CdCl_2$ + 0.5 mM SA, **k:** 200 μM $CdCl_2$ + 0.5 mM SA). The statistical significance is indicated above the bars, with capital letters representing concentration differences in the same day and numbers representing differences in days at the same concentration.

increased the H_2O_2 level on the 3rd and 5th days when compared to the control and 200 μM $CdCl_2$. This result contradicts the findings of Zhang et al. (40). SA treatment induced the H_2O_2 accumulation depending on the Cd intensity in the growth medium. High levels of H_2O_2 lead to oxidation of membrane proteins and inactivation of NR. Free radicals such as H_2O_2, O_2^-, or OH were determined to affect the membrane and membrane-bound structures in the ribosome and therefore influence the protein synthesis (41). In our study, this result can be supported by the decrease in the amount of protein observed, especially on the 5th day of 0.5 mM SA + 200 μM $CdCl_2$ treatment. Thus, in plants, SA has different effects on both stress and the development of damage.

High levels of H_2O_2 caused by high SA concentration lead to destruction in the structure of lipids, as well (42). MDA (lipid peroxidation) concentration is considered an indicator of lipid peroxidation caused by oxidative stress (42). MDA levels were determined to increase with the increase of Cd concentration (43). In this study, high $CdCl_2$ treatments increased the MDA level on the 1st and 3rd days; however, in comparison to the control, the maximum increase was detected when $CdCl_2$ and SA were co-administered on the 1st and 5th days. According to low cadmium concentrations, 0.5 mM SA decreased the toxic effect of high cadmium concentrations (100 and 200 μM $CdCl_2$) on 1st and 3rd days.

In this research, we found that $CdCl_2$ stress induced effects on total protein content, nitrogen metabolism, H_2O_2 level, and lipid peroxidation; 0.5 mM SA exerted little alleviative effect on H_2O_2 level and lipid peroxidation in leaves of tomato and 0.5 mM SA treatment demonstrated a negative effect on the 3rd and 5th days. However, the detailed mechanism for such results needs to be further studied. Apart from plant leaves, root systems also play an important part in resisting or tolerating environmental heavy metal stress. Many studies concerning the toxic effects of heavy metals on root systems were also conducted, and some indicated that heavy metals exerted more apparent inhibitory effects on root systems than on leaves (44). In this research, we implemented relevant experiments on plant leaves, and the data acquired from such experiments can serve as references for experiments concerning the effects of Cd stress and Cd + SA on the seedling root system.

Recent studies support that SA is a hormone-like signaling molecule that has an important role in regulating the plant defense responses against a variety of biotic and abiotic stresses. Low and high concentrations of SA and the duration of treatment affect the tolerance of plants against stress factors. The type of plant, stress factor, concentration of stress factor, duration of exposure to stress, and structure of the tissue or organ exposed to stress are also effective in this process. The data obtained from this study and previous studies demonstrate the necessity of many more research studies on SA signaling in plants.

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