

NITRIC OXIDE AND CALCIUM INDUCED PHYSIO-BIOCHEMICAL CHANGES IN TOMATO (*SOLANUM LYCOPERSICUM*) PLANT UNDER HEAT STRESS

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ABSTRACT

Temperatures above the threshold levels limit many physiological and biochemical processes required for plant survival. Therefore, the aim of the present experiment was to determine the individual and combined effects of sodium nitroprusside (SNP) and calcium (Ca) on the physiological attributes of tomato (*Solanum lycopersicum*) plants under heat stress (HS) and non-HS conditions. Heat stress significantly increased the chlorophyll (Chl) degradation, malondialdehyde (MDA), hydrogen peroxide (H₂O₂), proline (Pro) and glycinebetaine (GB) content, and decreased the photosynthetic pigment (Chl *a* and Chl *b*) and total soluble carbohydrate (TSC) content and the activities of Rubisco, carbonic anhydrase (CA), and nitrate reductase (NR). However, compared to the individual treatments, the combined application of SNP and Ca was more effective in subsiding the damaging effects of HS by reducing the accumulation of MDA and reactive oxygen species and by stimulating the biosynthesis of compatible solutes (Pro and GB), TSC, and photosynthetic pigments (Chl *a* and *b*), and also by enhancing the activities of Rubisco, CA, NR, superoxide dismutase, catalase, peroxidase, glutathione reductase, and ascorbate peroxidase.

KEYWORDS:

Heat stress, tomato, compatible solutes, Rubisco, nitric oxide, carbonic anhydrase

INTRODUCTION

Tomato (*Solanum lycopersicum*) is one of the important vegetable crops among the members of the nightshade family and is widely cultivated in many countries across the world. It is the second most-consumed and popular vegetable after potato, and a good source of nutrients and antioxidants, such as beta-carotene, lycopene, vitamin C, carotenoids, flavonoids, and hydroxycinnamic acid derivatives. Based on recent studies, it is considered as an anti-

cellular ageing and anti-cancer agent, as it provides the above-mentioned antioxidants [1-3]. Thus, it is very important to exploit different strategies to increase the production of tomato to meet the demand of the growing world population.

Due to the phenomenon of global warming, the increase in ambient temperature beyond a threshold causes an irreversible damage to the productivity of agricultural crops [4]. High temperature, particularly high nighttime temperature, limits the productivity of tomato, which is very sensitive to heat stress (HS) [5]. High temperature impairs pollen development and pollen viability by disrupting the carbohydrate metabolism, resulting in a decrease in tomato fruit set [6]. Also, HS disturbs proline translocation and hormonal balance in plants that reduce yield and fruit quality [7,8]. HS causes various alterations in plants at morphological, anatomical, physiological, biochemical, and molecular levels by altering protein structure, membrane integrity, RNA species, and the activity of enzymes, leading to a disturbance in the cellular homeostasis [9-11]. HS markedly limits photosynthesis, respiration, water balance, and membrane stability, and also disturbs primary and secondary metabolites in plants (10, 12, 13).

Abiotic stress leads to imbalanced generation of reactive oxygen species (ROS) that disturb metabolic homeostasis in plants, resulting in autocatalytic peroxidation of membrane lipids and pigments [9,12]. To counter oxidative damage, plants activate non-enzymatic and enzymatic detoxification system to maintain or reprogram the metabolic homeostasis [9]. The antioxidant enzymes, such as catalase (CAT), peroxidase (POD), superoxide dismutase (SOD), and ascorbate peroxidase (APX), as well as other macromolecules, such as proline (Pro), glycinebetaine (GB), carbohydrates play a key role in plants in coping with abiotic stress. Therefore, it is important to study the role of sodium nitroprusside [SNP, a source of nitric oxide (NO)] and calcium (Ca) in tolerance of tomato plants to HS.

Nitric oxide is a small diffusible and ubiquitous molecule that boosts the abiotic stress tolerance of plants by activating a series of physiological and biochemical mechanisms in plants. It plays a

significant role in the regulation of stomatal closure and photosynthesis [14-16]. Under different environmental stresses, NO develops a defense mechanism in plants to suppress the oxidative damage by regulating the antioxidant system and maintaining the ROS balance in plant cells [15,17]. Furthermore, NO regulates the intracellular Ca^{2+} homeostasis by activating the Ca^{+2} channels in plants [18]. The interrelationship between NO and Ca may further enhance the abiotic stresses tolerance of plants by maintaining the ion homeostasis, and regulating the antioxidant enzymes and expression of genes involved in tolerance [19,20]. During signal transduction, signaling through both NO and Ca involved in the plant growth and development as well as environmental stress responses may be activated [19]. Calcium is one of the essential plant nutrients, which acts as an intercellular secondary messenger in plants. It regulates physiological, biochemical, and molecular responses to various abiotic stresses [21-23]. Therefore, it is very interesting to study the interactive role of NO and Ca in signaling of plants under abiotic stress. The aim of the present study was to elucidate the combined role of NO and Ca in restoring the physiological and biochemical alterations caused by HS in tomato plants.

MATERIALS AND METHODS

Seeds of tomato (*Lycopersicon esculentum* L. var. Five Star F-1 Hybrid) were obtained from the local market of Riyadh, Saudi Arabia. Before sowing, the seeds were surface sterilized using 1% sodium hypochlorite for 10 min, followed by vigorous rinsing with sterilized double-distilled water (DDW). The seeds were germinated on two sheets of sterilized filter paper in Petri dishes (12-cm diameter). The following treatments were administered to the growing seedlings in different Petri dishes: (i) DDW, (T1: control); (ii) 0.1 mM SNP (T2); (iii) 20 mM CaCl_2 (T3); (iv) 0.1 mM SNP + 20 mM CaCl_2 (T4); (v) HS (42°C) (T5) (vi) 0.1 mM SNP + HS (T6); (vii) 20 mM CaCl_2 + HS (T7); (viii) 0.1 mM SNP + 20 mM CaCl_2 + HS (T8). Sodium nitroprusside ($\text{Na}_2[\text{Fe}(\text{CN})_5\text{NO}]\cdot 2\text{H}_2\text{O}$) was used as a NO donor. The Petri dishes were arranged in a sample-randomized design with a single factor with six replicates. All the Petri dishes were kept in a growth chamber at $25 \pm 3^\circ\text{C}$, 50–60% relative humidity, and $250 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ light provided under a 16/8-h light/dark cycle. After 25 days of germination, HS treatment was imposed on the tomato seedlings by placing the Petri dishes in an incubator at 42°C for 4 h.

Determination of physio-biochemical characteristics of plants.

Proline. For proline determination, Leaf

samples were ground in a solution of sulfosalicylic acid, ninhydrin and glacial acetic acid. After heating, the mixture was extracted with toluene. Pro concentration was measured spectrophotometrically ((SPEKOL 1500; Analytik Jena AG, Jena, Germany) at 520 nm using the ninhydrin method of Bates et al. [24].

Glycinebetaine. The content of glycinebetaine (GB) was estimated according to the method of Grieve and Grattan [25]. Dried leaf samples were finely ground with deionized water at 100°C for 60 min. The GB content was determined spectrophotometrically at 365 nm.

Malondialdehyde. The malondialdehyde (MDA) content was determined according to the method of Heath and Packer [26]. Plant leaves were ground in a solution comprising 10% trichloroacetic acid and 0.65% 2-thiobarbituric acid and then heated at 95°C for 60 min. The supernatant was collected after centrifuged of mixture at $10,000 \times g$ for 10 min. The supernatant was read at 532 and 600 nm against a reagent blank.

Hydrogen peroxide. Fresh leaf samples were ground in 0.1% (w/v) trichloroacetic acid. After centrifugation of the homogenate, the supernatant was added to a solution (10 mM potassium phosphate buffer (pH 7.0) and 1 M potassium iodide). The content of H_2O_2 was estimated as described by Velikova et al. [27] by calculating the comparison with a standard calibration curve plotted using known concentrations of H_2O_2 .

Chlorophylls. The chlorophylls (Chl) were extracted from fresh leaves of the plants from each treatment using the DMSO method as described by Barnes et al. [28]. The Chl content in each extract was measured using a UV-Vis spectrophotometer. The content of Chl was calculated using the following formula:

$$\text{Chl } a = 14.85 A_{664.9} - 5.14 A_{648.2}$$

$$\text{Chl } b = 25.48 A_{648.2} - 7.36 A_{664.9}$$

Chl degradation was expressed by the ratio between the absorbance at 435 and 415 nm (A_{435}/A_{415}), as suggested by Ronen and Galun [29].

Total soluble carbohydrate. Total soluble carbohydrate (TSC) concentration was estimated by determining the absorbance at 490 nm, as described by Dubois et al. [30], using glucose as a standard. TSC was expressed as mg g^{-1} dry weight (DW).

Calcium content. To measure the Ca content, leaf tissue was digested according to the method of Zheljzkov and Nielson [31] as modified by Hseu [32]. The leaf tissue (0.5 g) was digested in a tube containing 2:1 concentrated nitric acid: perchloric

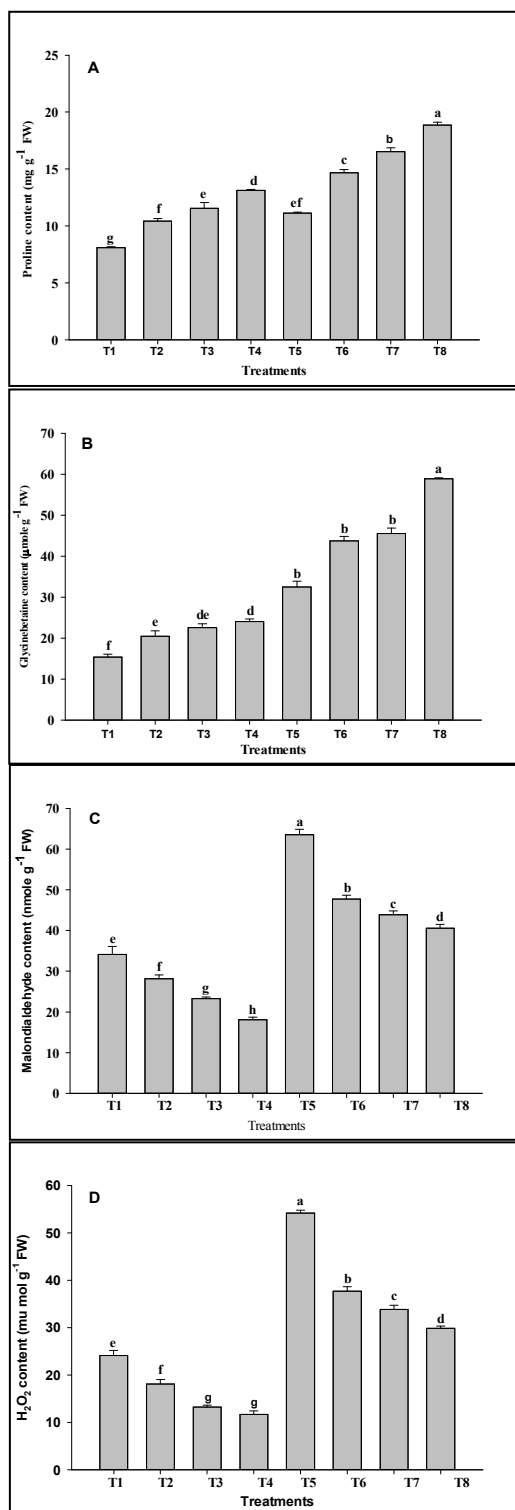


FIGURE 1

Effect of SNP and Ca treatment on (A) proline, (B) glycinebetaine, (C) malondialdehyde and (D) hydrogen peroxide content of tomato plant under HS. Tomato plants treated with (T1) DDW (control); (T2) 0.1 mM SNP; (T3) 20 mM CaCl₂; (T4) 0.1 mM SNP+20 mM CaCl₂; (T5) HS (42 °C); (T6) 0.1 mM SNP + HS; (T7) 20 mM CaCl₂+HS; (T8) 0.1 mM SNP+20 mM CaCl₂ + HS

acid. After complete digestion, 5 mL of 1% HNO₃ was added to each sample and filtered through Whatman No. 42 filter paper and Millipore filter paper with <0.45 µm pore size. The Ca content was determined using an atomic absorption spectrometer (Model 300, Perkin-Elmer, Waltham, MA, USA).

Determination of enzyme activities. Carbonic anhydrase (CA; EC 4.2.1.1) activity was determined using the method described by Dwivedi and Randhawa [33], and presented as µmol CO₂ kg⁻¹ (FW) s⁻¹.

Rubisco (EC 4.1.1.39) activity was determined by measuring NADH oxidation at 340 nm [34]. Leaf samples from each treatment were homogenized using a chilled mortar and pestle with ice-cold extraction buffer (0.05 M MgCl₂, 250 mM Tris-HCl, pH 7.8), 2.5 mM EDTA, and 37.5 mg DTT. The supernatant obtained after centrifugation of the extract at 10,000 × g for 10 min at 4°C was used for the enzyme assay. NADH oxidation was initiated by adding the enzyme extract and 0.2 mM ribulose-1,5-bisphosphate (RuBP) in a reaction mixture (100 mM Tris-HCl (pH 8.0), 40 mM NaHCO₃, 10 mM MgCl₂, 0.2 mM NADH, 4 mM ATP, 0.2 mM EDTA, 5 mM DTT, 1 U of glyceraldehyde 3-phosphodehydrogenase, and 1 U of 3-phosphoglycerate kinase), and the absorbance was subsequently determined for 1 min after stopping the reaction. The enzyme activity was expressed as µmol CO₂ fixed min⁻¹ mg⁻¹ protein. The protein content was measured according to Bradford [35].

Nitrate reductase (NR; EC 1.6.6.1) activity was determined by the intact tissue method described by Jaworski [36]. Fresh leaf samples were incubated in a mixture containing 2.5 mL phosphate buffer (pH 7.5), 0.2 M potassium nitrate, and 5% isopropanol. The absorbance was read at 540 nm after adding 1% sulphanilamide and 0.2% *N*-1-naphthylethylene-diamine dihydrochloride, and compared using a calibration curve. The activity of NR was expressed as nmol NO₂ g⁻¹ FW h⁻¹.

To measure the activities of ROS scavenging enzymes, leaf samples from each treatment were ground in pre-chilled mortar and pestle with extraction buffer (0.5% Triton X-100 and 1% polyvinylpyrrolidone in 100 mM potassium phosphate buffer, pH 7.0). Ascorbate (1 mM) was added to this mixture for assay of ascorbate peroxidase (APX). After centrifugation of the homogenates at 15,000 × g for 15 min at 4°C, the supernatant was stored for the enzymatic assays as described below.

Superoxide dismutase (SOD; EC 1.15.1.1) activity was measured using the photochemical method as described Giannopolitis and Ries [37]. The enzyme extract was added in a reaction mixture containing 50 mM nitroblue tetrazolium (NBT), 1.3 mM riboflavin, 13 mM methionine, 75 µM EDTA, and 50 mM phosphate buffer (pH 7.8). After

irradiation of the reaction mixture with fluorescent light at $75 \mu\text{M m}^{-2} \text{s}^{-1}$ for 15 min, the absorbance was read at 560 nm against a blank (non-irradiated reaction mixture). One unit of SOD activity was defined as the amount of enzyme that inhibited 50% of NBT photoreduction.

The method of Aebi [39] was used to determine the catalase (CAT) (EC 1.11.1.6) activity. H_2O_2 decomposition was determined as the decline in absorbance at 240 nm. The reaction mixture consisted of 50 mM phosphate buffer (pH 7.8) and 10 mM H_2O_2 .

For the assay of Peroxidase (POD; EC 1.11.1.7) activity, 5 mL reaction mixture containing phosphate buffer (pH 6.8), 50 M pyrogallol, 50 mM H_2O_2 and the enzyme extract was incubated at 25°C for 5 min. The reaction was stopped by adding 0.5 mL of 5% (v/v) H_2SO_4 . The production of purpurogallin was determined spectrophotometrically at 420 nm (Chance and Maehly [40]).

The method of Foyer and Halliwell [41] was used to determine the activity of glutathione reductase (GR; E.C. 1.6.4.2). The assay mixture consisted 50 μL of the enzyme extract, 100 mM phosphate buffer (pH 7.8), 0.1 μM EDTA, 0.05 mM NADPH, and 3.0 mM oxidized glutathione in a total volume of 1.0 mL. The NADPH oxidation rate was calculated by measuring the absorbance at the time of H_2O_2 addition and 1 min thereafter. The difference in two absorbance was divided by the NADPH molar extinction coefficient (6.22 mM cm^{-1}) and the enzyme activity was expressed as μM of NADPH $\text{min}^{-1} \text{ mg}^{-1}$ protein.

The method of Nakano and Asada [42] was used to determine the activity of ascorbate peroxidase (APX; EC 1.11.1.11). The activity was assayed by adding the enzyme extract to the reaction mixture containing 50 mM potassium phosphate (pH 7.0), 0.1 mM EDTA, 0.1 mM H_2O_2 , and 0.5 mM ascorbate. The H_2O_2 -dependent oxidation of ascorbate was followed by measuring the decrease in the absorbance at 290 nm (the extinction coefficient used was $2.8 \text{ mM}^{-1} \text{ cm}^{-1}$).

Statistical analysis. The data were analyzed statistically using SPSS statistical software and presented as means \pm standard error. The means were statistically compared using ANOVA, followed by Duncan's multiple-range test (DMRT) at the level of $p < 0.05\%$.

RESULTS

Pro and GB accumulation. After exposure to HS, the plants exhibited a significant increase in Pro and GB content in their leaves compared to the control (Fig.1 A and B). The plants exposed to HS had 37.40 and 110.75% higher Pro and GB content, respectively compared to their respective controls.

However, the combined application of SNP and Ca further enhanced the accumulation of Pro and GB significantly in comparison to the application of SNP and Ca alone. Under HS stress, the application of SNP along with Ca enhanced the Pro content by 69.25% and GB by 81.08% compared to the respective controls.

Lipid peroxidation and H_2O_2 content. The adverse effect of HS was evaluated on the basis of lipid peroxidation by recording the MDA content in the leaves, and the membrane injury was examined by measuring the H_2O_2 content (Fig. 2 C and D). After the exposure to HS, the plants showed about 2-fold increase in MDA and H_2O_2 compared to their respective controls. However, the application of SNP and Ca, individually, was associated with lower MDA and H_2O_2 content in the leaves than the combined application. The combined application of SNP and Ca decreased MDA by 36.13% and H_2O_2 by 44.82% compared to their content after HS application.

Accumulation and degradation of chlorophylls. The content of Chl *a* and *b*, was severely affected in plants exposed to HS (Fig. 2 A). In addition, Chl degradation was recorded more in HS exposed plants than in the control plants (Fig. 2B). After exposure to HS, the plants exhibited 39.37 and 48.23% lower levels of Chl *a* and *b*, respectively, and 163.21% higher Chl degradation than their respective controls. However, the combined application of SNP and Ca improved the synthesis of the photosynthetic pigments and suppressed the degradation of Chl as compared to the application of these chemicals individually. The application of SNP in combination with Ca enhanced the Chl content by 53.51% and Chl *b* by 173.38%, and decreased Chl degradation by 50.35%, in comparison to the HS exposed plants.

Total soluble carbohydrate content. The application of SNP and Ca individually as well as in combination significantly increased the concentration of TSC in the leaves relative to the control plants (Fig. 2 D). The lowest value for TSC content was observed in the plants exposed to HS treatment. Under HS conditions, the TSC level was recorded to be higher in the combined treatment of SNP and Ca than in the treatments with these chemicals individually. The combined application of SNP and Ca increased the TSC content by 32.85% compared to the content in the HS exposed plants.

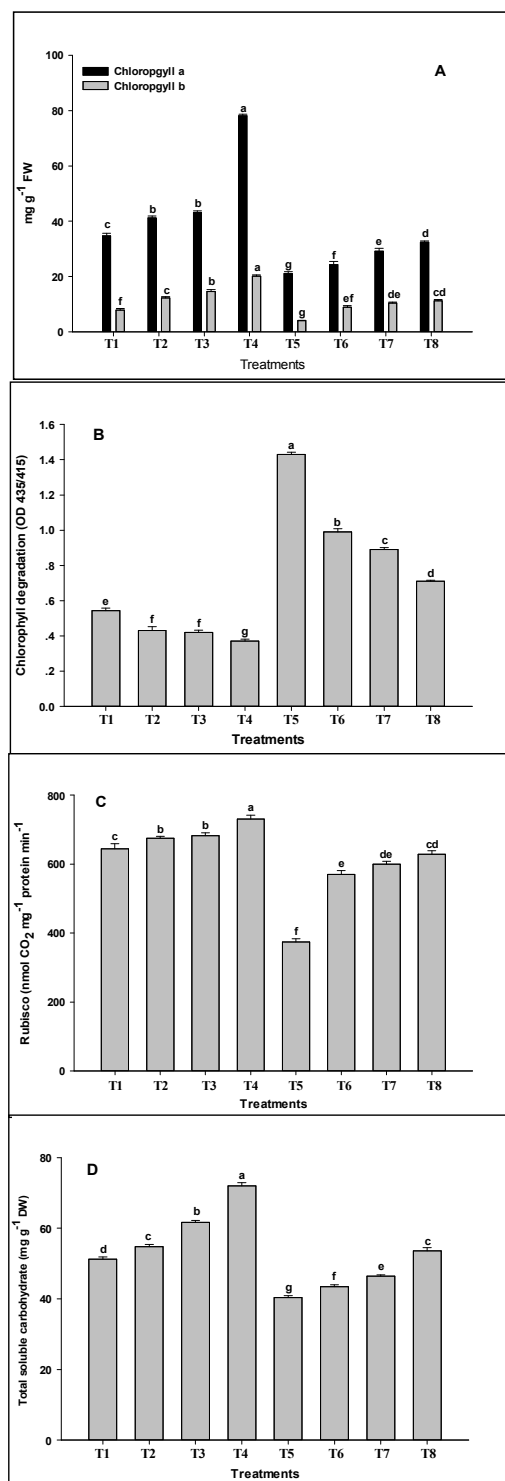


FIGURE 2

Effect of SNP and Ca treatment on (A) Chlorophyll a and b, (B) Chlorophyll degradation, (C) Rubisco activity and (D) Total soluble carbohydrate content of tomato plant under HS. Tomato plants treated with (T1) DDW (control); (T2) 0.1 mM SNP; (T3) 20 mM CaCl₂; (T4) 0.1 mM SNP+20 mM CaCl₂; (T5) HS (42 °C); (T6) 0.1 mM SNP + HS; (T7) 20 mM CaCl₂ +HS; (T8) 0.1 mM SNP+20 mM CaCl₂ + HS

Enzymes activity. Under HS condition, a significant decrease in the activity of Rubisco, CA, and NR was observed in comparison to control plants (Figs. 2 C and 3 A, B). However, the application of SNP and Ca alone as well as in combination enhanced the activity of these enzymes under the stress and non-stress conditions. The application of SNP and Ca together increased the activities of Rubisco by 68.09%, CA by 18.88%, and NR by 17.62% over the activities in the HS exposed plants.

Calcium content. The HS exposed plants exhibited the lowest content of Ca (Fig. 3C). However, the application of SNP and Ca individually as well as in combination significantly enhanced the content of Ca under both the stress and non-stress conditions. The content of Ca was noted to be higher in the plants treated with SNP and Ca together than in the plants treated with these chemicals individually. The combined application of SNP and Ca increased the Ca content by 82.41% over the Ca content in the HS exposed plants.

Activities of antioxidant enzymes. All the treatments significantly enhanced the activities of the antioxidant enzymes, SOD, CAT, POS, GR, and APX relative to their respective controls, although the effects were most notable with the combined application of SNP and Ca under both the stress and non-stress conditions (Fig. 4 A, B, and C). The combined application SNP and Ca increased the activity of SOD, POD, GR, and APX by 42.23, 17.00, 52.42, and 19.65%, respectively compared to that in the HS exposed plants. However, the combined application of SNP and Ca showed similar CAT activity as observed in the HS exposed plants.

DISCUSSION

It is well established that both Pro and GB are key compatible organic solutes that play an important role in the osmotic adjustments by maintaining the osmotic potential within the cells [23]. In the present study, both the solutes, Pro and GB, increased relative to the control when tomato plants received the doses of SNP and Ca, either in combination or individually, under stress and non-stress conditions (Fig. 1 A and B). However, the highest accumulation of Pro and GB occurred when the plants were subjected to the combined dose of SNP and Ca. Hyper-accumulation of both the solutes might have caused protection from the oxidative damage induced by HS. The accumulation of GB might have improved the resistance of photosystem (PS)II to HS by stimulating photoinhibition [43] and also by repairing the damage of PSII [44]. In addition, the

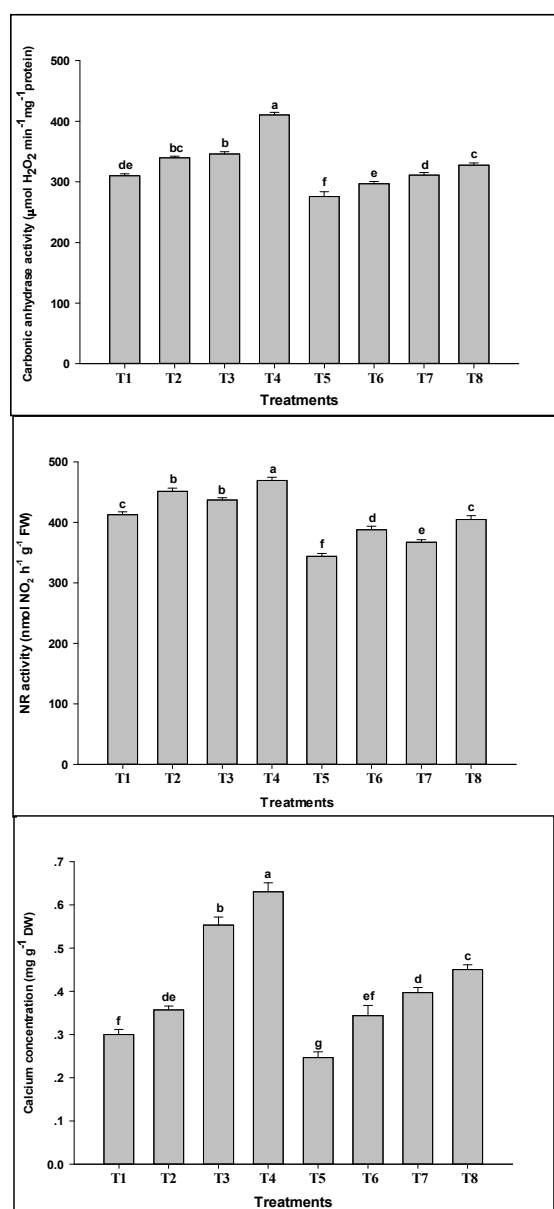


FIGURE 3

Effect of SNP and Ca treatment on (A) carbonic anhydrase activity (B) nitrate reductase activity, (C) calcium content of tomato plant under HS. Tomato plants treated with (T1) DDW (control); (T2) 0.1 mM SNP; (T3) 20 mM CaCl₂; (T4) 0.1 mM SNP+20 mM CaCl₂; (T5) HS (42 °C); (T6) 0.1 mM SNP + HS; (T7) 20 mM CaCl₂ +HS; (T8) 0.1 mM SNP+20 mM CaCl₂ + HS

accumulation Pro might have been the reason for the HS tolerance of the plants by providing energy and nitrogen for growth and development and also by stabilizing the membranes and proteins. It can also act as an antioxidant to scavenge the reactive oxygen species. Thus, we postulate that application of SNP in combination with Ca enhanced Pro and GB content, which was responsible for the improved tolerance to stress, as manifested in the increase in Ca content and the activities of Rubisco, CA, and NR (Figs. 2A and 3A, B, C).

In general, ROS metabolically produced in the plant cells under environmental stress, and disturbs the plant metabolism by reacting with a large variety of macromolecules, such as DNA, RNA, proteins, carbohydrates, and amino acids [23,45]. Therefore, it is important to limit the production of ROS and scavenge them once they are produced. In the present study, the levels of MDA and H₂O₂ were significantly higher in the plants exposed to HS (Fig. 1 C and D). However, the application of SNP in combination with Ca was effective in removal of ROS by enhancing the organic solutes and antioxidant enzymes. Nitric oxide is also involved in the detoxification of ROS by interaction with superoxide and regulation of the activities of antioxidant enzymes [46,47].

Chlorophylls are important components in the process of photosynthesis. Heat stress limits the photosynthetic capacity of plants by affecting the synthesis of pigments. In the present study, HS severely affected the Chl *a* and *b* synthesis and accelerated the Chl degradation (Fig. 2 A and B). These results are in agreement with the findings of Nankishore and Farrell [48]. Interestingly, we observed that the plants exhibited higher levels of Chl *a* and *b*, and lower levels of Chl degradation when they received SNP and Ca together under the HS condition (Fig. 2 A and B). It may be due to the roles of Ca and NO: Ca limits photooxidation of organic molecules and maintains the membrane integrity [49], and NO regulates the iron homeostasis and enhances the iron transport in cells, thereby accelerating the synthesis of photosynthetic pigments and inhibiting the degradation of Chls [50].

HS significantly disturbs the lipid and carbohydrate metabolism in plants. The plants exposed to HS showed a significant decrease in the TSC content (Fig. 2 D). This result substantiates the findings of Jie et al. [51]. The degree of decrease in the TSC content in leaves under HS might be due to the inhibition of photosynthesis caused by the reduction of Chls, activity of Rubisco and CA, degradation of Chls (Figs. 2 A-D and Fig. 3 A), and also due to the changes in the carbohydrate-metabolizing enzymes [51]. However, the application of SNP and/or Ca substantially increased the content of TSC under both stress and non-stress conditions (Fig. 2D). The level of increase in the TSC content in the leaves of tomato might be due to the role of NO and Ca; NO, as a signaling molecule, activates many enzymes, and Ca regulates the carbohydrate metabolism by activating the carbohydrate-metabolizing enzymes [23, 52]. The increased levels of TSC could be helpful in maintaining the osmotic balance, resulting in an increased resistance to HS.

In the present study, the activities of Rubisco, CA, and NR were severely affected by the exposure to HS (Figs. 2 C and 3 A, B). The impairment of the activities of these enzymes might be due the

accumulation of ROS that leads to the alteration of proteins and plant metabolism.

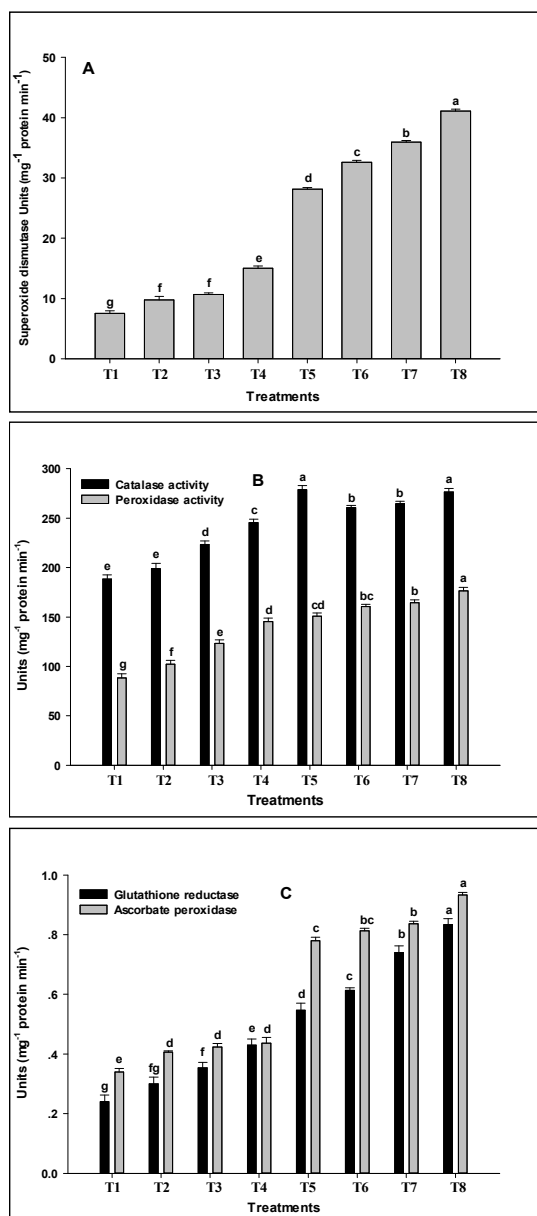


FIGURE 4

Effect of SNP and Ca treatment on (A) superoxide dismutase, (B) catalase and peroxidase activity (C) glutathione reductase and ascorbate peroxidase activity of tomato plant under HS. Tomato plants treated with (T1) DDW (control); (T2) 0.1 mM SNP; (T3) 20 mM CaCl₂; (T4) 0.1 mM SNP+20 mM CaCl₂; (T5) HS (42 °C); (T6) 0.1 mM SNP + HS; (T7) 20 mM CaCl₂ +HS; (T8) 0.1 mM SNP+20 mM CaCl₂ + HS

Also, HS reduces the availability of CO₂, resulting in the decreased Rubisco activity that limits the photosynthesis [53]. However, the application of SNP and/or Ca significantly enhanced the activity of these enzymes, but the maximum enhancement was seen with the combined application of SNP and Ca; therefore, these results suggest that SNP and Ca have

interrelated metabolic roles in plant growth and development. The improved CA activity due to the combined application of SNP and Ca might improve the acid-base as well as ion homeostasis, and facilitate the diffusion of inorganic carbon in the cells and reversible hydrogenation of CO₂ in plants, thereby, maintaining its continuous supply to Rubisco leading to improved net photosynthetic rate [23]. Under the HS conditions, the NR activity was also found to be decreased. This result strengthens the finding of Magalhães et al. [54]. The NR activity increased relative to the control and HS treatments when the plants received SNP and/or Ca. Khan et al. [22] reported that the application of Ca enhanced the NR activity and improved the stress tolerance of plants. All these findings indicate the improved resistance of tomato plants to HS.

The result obtained in the present study reveals that HS decreased the content of Ca in the tomato leaves (Fig. 3C). This result is consistent with the findings of Giri [55]. However, the application of SNP and Ca alone increased the Ca content in leaves relative to the control, although the content was more when SNP and Ca were administered in combination. This reveals that SNP and Ca might have synergistic effects in mitigation of HS. The increased accumulation of Ca might be involved in HS-signal transduction to regulate the expression of HS-tolerance genes [56].

It is important to limit the generation of ROS, scavenge them once formed, and restore the impairment induced by ROS in plants under abiotic stress. In the present study, HS-induced damage in the form of MDA and ROS (H₂O₂) generation, and their scavenging was done by the activity of the antioxidant enzymes, such as SOD, CAT, POS, GR, and APX (Fig. A,B,C). Interestingly, under stress, the activities of these enzymes were recorded to be the highest in plants receiving combined application of SNP and Ca. The improved accumulation of Ca was positively correlated with the decrease in MDA and H₂O₂ content by increasing the activity of antioxidant enzymes. These results validate the findings of Siddiqui et al. [23]. However, the enhanced activities of these enzymes in response to the combined application of SNP and Ca suggest that both of these had synergistic effects on the plants.

CONCLUSION

Overall, the results of the present study indicate that synthesis of photosynthetic pigments and Chl degradation were affected by HS by affecting the activities of Rubisco and CA. However, the application of Ca had a synergistic influence on the plant physiological mechanisms when supplied in combination with SNP. An increase in the accumulation of Ca was observed in plants receiving SNP. The obtained data reveal that the combined

application of SNP and Ca decreased the accumulation of MDA and H₂O₂ and increased the accumulation of compatible solutes (Pro and GB) as well as the activity of NR in tomato plants. Application of SNP and Ca in combination was found to be more effective, relative to the individual treatments, in the mitigation of oxidative damage through elevated ROS scavenging by the antioxidant enzymes under HS. However, considerable studies at molecular and physiological levels are needed to determine the interactive effects of SNP and Ca in plants under HS.

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