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(Research Paper)

The modulatory role of sodium nitroprusside (SNP) in maintaining oxygen-evolving complex functionality in tomato under heat stress

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Abstract

Heat stress severely impairs photosynthetic capacity and induces oxidative damage in tomato plants, threatening agricultural productivity. This study investigated the protective role of sodium nitroprusside (SNP) against heat stress-induced damage, with a specific focus on oxygen-evolving complex (OEC) functionality. Under controlled environmental conditions, tomato plants were subjected to heat stress at 45 °C, with or without pretreatment with 100 µM SNP. Heat stress drastically reduced net photosynthesis (PN) by 47.07% and significantly decreased the performance index (PIABS) by 88.9%. Furthermore, heat stress increased H_2O_2 levels by 56.23% and reduced the activities of CAT, POD, SOD, and APX by up to 43.7%. However, SNP pretreatment alleviated these adverse effects by limiting the reduction in PN to 20.13% and increasing antioxidant enzyme activities by up to 161.2% compared with heat-stressed plants. Moreover, SNP treatment significantly suppressed the K-band transient, suggesting the preservation of OEC functionality under heat stress. This response, together with improved photosynthetic performance and antioxidant capacity, indicates that SNP treatment contributed to maintaining Photosystem II donor-side activity under heat stress. Overall, SNP treatment effectively alleviated heat stress-induced physiological and photosynthetic damage and may contribute to improved heat tolerance in tomato plants.

Keywords: Heat stress, Photosynthesis, Oxidative stress, OEC, Sodium nitroprusside, Tomato.

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Introduction

Global agriculture is currently undergoing a profound shift due to the climate crisis and the increasing frequency of extreme thermal events (Benga & Wakweya, 2025). According to recent statistics from the Food and Agriculture Organization (FAO), rising global temperatures are projected to reduce crop yields significantly, threatening food security on a global scale (Dossa et al., 2025). Among economically significant horticultural crops, the tomato (Lycopersicon esculentum Mill.) is one of the main components of human nutrition (Ali et al., 2021). However, as temperatures rise beyond the optimal physiological threshold, the complex biochemical processes of plant growth and productivity are negatively affected. Thermal stress triggers a series of detrimental effects that extend from growth and development to the disruption of cellular organelles, particularly the chloroplasts, which are the primary sites of photosynthesis (Graci & Barone, 2024).

The photosynthetic apparatus is highly sensitive to thermal fluctuations and plays an important role in plant survival under environmental stresses (Zahra et al., 2023). Photosystem II (PSII) is the most vulnerable component of the photosynthetic electron transport chain, particularly the Oxygen-Evolving Complex (OEC) located on the luminal side of the thylakoid membrane (Gupta, 2020). The OEC is responsible for water photolysis, a process that provides the electrons required for the reduction of P680 and the subsequent generation of chemical energy. This process is driven by a unique manganese-calcium cluster, Mn4CaO5, which operates through the highly regulated Kok Cycle (S0 to S4 states) (Gupta, 2020). The stability of this cluster is highly dependent on its association with a group of extrinsic proteins, including PsbO, PsbP, and PsbQ, which protect the metal center and facilitate efficient proton release (Sasi et al, 2018). Under conditions of severe heat stress, the thermodynamic stability of these protein-lipid-cofactor interactions is often lost, leading to the dissociation of the extrinsic proteins and the subsequent inactivation of the water-splitting mechanism (Allakhverdiev et al., 2008). Damage to OEC not only limits the supply of electrons but also triggers the formation of reactive oxygen species (ROS), leading to the oxidative degradation of the D1 protein and a phenomenon known as photoinhibition (Billah et al., 2024).

To mitigate these adverse effects, plants have evolved complex signaling networks to sense and respond to thermal signals. Nitric oxide (NO), a small, gaseous molecule, plays a central role in these stress-response pathways (Wani et al., 2021). NO can penetrate biological membranes rapidly, allowing it to exert systemic regulatory effects in different tissues. The role of NO in plant thermotolerance involves both antioxidant regulation and structural protein stabilization (Naaz et al., 2025). One of the most significant mechanisms of NO action is S-nitrosylation, a post-translational modification where NO is covalently attached to the thiol (-SH) groups of cysteine residues in target proteins (Wani et al., 2021). It is hypothesized that S-nitrosylation may play a crucial role in maintaining the structural integrity of the PSII-OEC assembly by stabilizing the extrinsic proteins against heat-induced dissociation (Yu et al., 2020). Furthermore, NO is known to upregulate the activity of key antioxidant enzymes, such as superoxide dismutase (SOD) and ascorbate peroxidase (APX), thereby reducing the oxidative stress caused by the accumulation of ROS during heat stress (Wani et al., 2021).

While the general role of NO in abiotic stress mitigation has been documented in several major crops (Dehbozorgi et al., 2025; Khalilpoor & Jafarinia, 2017; Mirzaei Chegeni et al., 2024), a significant gap remains in our understanding of the specific responses of the OEC to heat stress in tomato. Previous studies have demonstrated that SNP enhances heat tolerance mainly by improving antioxidant defense, maintaining membrane stability, regulating osmolyte accumulation, and preserving photosynthetic performance. However, in tomato, most investigations have primarily focused on general physiological and biochemical responses, such as gas exchange, chlorophyll content, antioxidant metabolism, and plant growth. Several studies have demonstrated that exogenous application of SNP, as a nitric oxide donor, enhances plant tolerance to heat stress by improving antioxidant defense, maintaining photosynthetic performance, and reducing oxidative damage (Hasanuzzaman et al., 2012; Fancy et al., 2017; Siddiqui et al., 2017). In tomato, previous studies have mainly focused on physiological and biochemical traits, including photosynthetic pigments, antioxidant enzymes, gas exchange, and general chlorophyll fluorescence responses. However, the effects of SNP on the functional status of the OEC, particularly through K-band analysis combined with comprehensive OJIP-derived parameters and gas-exchange measurements, remain poorly understood.

The present study aimed to evaluate the effects of SNP on photosynthetic performance in tomato plants exposed to heat stress, with particular emphasis on OEC functionality. The novelty of this study lies in the integrated assessment of K-band

analysis, detailed OJIP-derived parameters, gas-exchange characteristics, antioxidant enzyme activities, and oxidative stress markers to provide a comprehensive evaluation of the physiological responses associated with SNP treatment under heat stress. Rather than inferring direct structural protection of the OEC or maintenance of the catalytic activity of the manganese cluster, this study evaluates physiological evidence suggesting the maintenance of OEC functionality together with improved photosynthetic performance in response to SNP treatment.

Materials and Methods

Plant Material and Growth Conditions

Tomato seeds (*Lycopersicon esculentum* Mill.) were obtained from the Seed and Plant Improvement Institute of Iran (SPII). Prior to sowing, the seeds were surface-sterilized by immersion in 70% ethanol for 2 min, followed by 6% sodium hypochlorite for 5 min, and then thoroughly rinsed with distilled water. The sterilized seeds were sown in plastic pots (20 cm in diameter × 25 cm in height) containing a peat moss and perlite mixture (2:1, v/v). All pots were maintained in a controlled-environment growth chamber set at 25 ± 2 °C, 60 ± 5% relative humidity, and a 16 h light/8 h dark photoperiod. Two weeks after sowing, seedlings were thinned to one healthy plant per pot. Plants were irrigated twice weekly with half-strength Hoagland nutrient solution throughout the experiment.

Sodium Nitroprusside and Heat Stress Treatments

Sodium nitroprusside (SNP, Sigma-Aldrich) was used as the nitric oxide (NO) donor. A preliminary experiment, together with evidence from previous studies, was used to identify an effective SNP concentration for the main experiment. Tomato plants were evaluated following application of 0, 50, 100, and 150 µM SNP, and 100 µM was selected because it produced the most favorable physiological response within the tested concentration range. It should be noted that the purpose of this preliminary experiment was to identify an effective concentration rather than to establish a complete dose–response relationship. SNP was prepared at 100 µM (24.2 mg L^{-1} in distilled water) and applied as a foliar spray twice per week until complete leaf coverage was achieved. Thirty days after sowing, the plants were divided into two groups. The first group was maintained under normal environmental conditions and served as the control, whereas the second group received foliar application of 100 µM SNP. Thirty days after the first SNP application (60 days after sowing), plants from both groups were transferred daily at 09:00 to a second growth chamber for heat treatment. Except for temperature, all environmental conditions, including light intensity, photoperiod, and relative humidity, were identical to those of the control chamber. The chamber temperature was gradually increased from 25 °C to 45 °C over a period of 1 h (09:00–10:00), after which plants were maintained at 45 °C until 17:00. At the end of each daily treatment, plants were returned to the original growth chamber maintained at 25 °C. This heat treatment was repeated for ten consecutive days. Tomato leaves were harvested immediately after completion of the heat treatment (70 days after sowing). Leaf samples were either used directly for physiological measurements or stored appropriately for subsequent biochemical analyses. The experimental design consisted of four treatment groups: (1) Control, (2) SNP, (3) Heat stress, and (4) Heat stress + SNP. The experiment was arranged as a completely randomized design (CRD) with three biological replicates.

Measurement of photosynthetic and dark respiration rates

Net photosynthetic (P_N) and dark respiration (R_d) rates were determined using a LeafLab-2 gas-phase oxygen electrode system (Hansatech Instruments Ltd., Lynn, UK) equipped with an LD2/3 leaf-disc chamber and a Clark-type oxygen electrode. Fully expanded leaves were sampled from the youngest fully expanded leaf of each plant. Leaf discs (10 mm in diameter) were excised with a cork borer, carefully avoiding the main veins, and immediately placed into the measurement chamber. The discs were positioned on a stainless-steel sample holder over a moist capillary mat to minimize water loss during the measurements. Before each measurement, the chamber atmosphere was flushed with fresh ambient air using a gas-tight syringe, and the oxygen electrode was calibrated following the manufacturer's recommendations. The measurement chamber was maintained at 25 ± 1 °C using a thermostatically controlled circulating water bath. Dark respiration (R_d) was measured first by incubating the leaf discs in complete

darkness until a stable oxygen consumption rate was obtained. The same samples were then exposed to saturating white light provided by the LS2 light source at a photosynthetic photon flux density (PPFD) of approximately 1200 µmol photons m^{-2} s^{-1}. After reaching a steady state, the oxygen evolution rate was recorded and used to calculate the net photosynthetic rate (P_N). Both PN and Rd were expressed as µmol O_2 m^{-2} s^{-1}. The gross photosynthetic rate (P_G) was calculated as follows:

$$P_G = P_N + R_d \quad \text{(Eq. 1)}$$

Where P_G is the gross photosynthetic rate, P_N is the net photosynthetic rate, and R_d is the dark respiration rate.

Chlorophyll *a* fluorescence analysis

Fast chlorophyll *a* fluorescence transients (OJIP) were measured using a Handy PEA fluorometer (Hansatech Instruments Ltd., Lynn, UK). Fully expanded leaves were dark-adapted for 30 min before measurements using leaf clips. Chlorophyll *a* fluorescence was induced by exposure to a saturating red light pulse of 3000 µmol photons m^{-2} s^{-1}, and fluorescence signals were recorded from 50 µs to 1 s (Strasser et al., 2004). The OJIP fluorescence transients were analyzed according to the JIP-test to calculate the normalized variable fluorescence at the K-step (ΔW_{OJ}), which was used as an indicator of changes in OEC functionality under heat stress. The K-band was calculated as $W_{OJ} = (F_t - F_0)/(F_J - F_0)$, where F_0 is the minimum fluorescence (50 µs), F_J is the fluorescence intensity at the J-step (2 ms), and F_t is the fluorescence intensity at time t. The K-band was subsequently expressed as $\Delta W_{OJ} = W_{OJ}$ (treatment) − W_{OJ} (control), which was used as an indicator of alterations in the functional integrity of the oxygen-evolving complex (OEC). To provide a comprehensive evaluation of PSII performance, additional JIP-test parameters were also calculated, including the specific energy fluxes per active reaction center (ABS/RC, TR_0/RC, ET_0/RC, and DI_0/RC), the phenomenological energy fluxes per excited cross-section (ABS/CS, TR_0/CS, ET_0/CS, and DI_0/CS), the density of active reaction centers (RC/CS), the performance index on an absorption basis (PI_{ABS}) and the photochemical (k_P) and non-photochemical (k_N) de-excitation rate constant (Tsimilli-Michael, 2020). These parameters were interpreted together with K-band analysis to evaluate the effects of heat stress and SNP treatment on PSII photochemical performance. Selected JIP-test parameters, together with their definitions and calculation methods, are presented in Table 1.

Table 1. Abbreviations, formulae, and glossary of selected JIP parameters.

Parameter	Explanation
F_0	Minimal fluorescence, when all PSII RCs are open
$F_{300\mu s}$	Fluorescence intensity at 300 µs
F_J	Fluorescence intensity at the J-step (2 ms) of OJIP
F_I	Fluorescence intensity at the I-step (30 ms) of OJIP
F_P (F_M)	Maximal recorded fluorescence intensity at the peak P of OJIP. All PSII RCs are closed.
Ft	Fluorescence at time t after onset of actinic illumination
$V_K = (F_K - Fo)/(Fm - Fo)$	Relative variable fluorescence intensity at the K-step
$ABS/RC = M_O (1/V_J) (1/\varphi Po)$	Absorption flux (for PSII antenna chlorophylls) per reaction center (RC)
$TR_O/RC = M_O (1/V_J)$	Trapped energy flux (leading to Q^A reduction) per reaction center RC
$ET_O/RC = M_O (1/V_J) \psi o$	Electron transport flux per PSII reaction center (RC)
$DI_O/RC = (ABS/RC) - (TRo/RC)$	Dissipated energy flux per reaction center (RC)
$ABS/CS = F_{400\mu s}$	Absorption flux per CS
$TR_o/CS = \varphi P_o (ABS/CS)$	Trapped energy flux per CS
$DI_o/CS = (ABS/CS_m) - (TR_o/CS)$	Dissipated energy flux per CS
$ET_o/CS = \varphi E_o (ABS/CS)$	Electron transport flux per CS
$RC/CS = F_{400\mu s}/ [(M_0/V_J)/ [1- (F_0/F_M)]$	Density of active reaction centers per CS
$k_N = (ABS) \times k_F \times (1/F_M)$	Non-photochemical de-excitation rate constant
$k_P = (ABS) \times k_F \times (1/F_0 - 1/F_M) = k_N \times (F_V/F_0)$	Photochemical de-excitation rate constant
$PI_{ABS} = RC/ABS [\varphi Po/(1-\varphi Po)] [\psi o/(1-\psi o)]$	The performance index for energy conservation from photons absorbed by photosystem II until the reduction of inter-system electron acceptors

Oxidative stress markers

Hydrogen peroxide (H_2O_2) concentration was determined according to Velikova et al. (2000). Briefly, 1 g of fresh leaf tissue was homogenized in 5 mL of 0.1% (w/v) trichloroacetic acid (TCA). The homogenate was centrifuged at 16,000 × g for 15 min, after which 500 µL of the supernatant was mixed with 500 µL of 10 mM potassium phosphate buffer (pH 7.0) and 1 mL of 1 M potassium iodide (KI). The absorbance of the reaction mixture was

measured at 390 nm, and H_2O_2 concentration was calculated from a standard curve. The results were expressed as μmol g^{-1} fresh weight (FW).

Malondialdehyde (MDA) content was determined following the method of Heath & Packer (1968). Briefly, 500 mg of fresh leaf tissue was homogenized in 5 mL of 0.1% (w/v) TCA and centrifuged at 10,000 × g for 20 min. An aliquot (500 μL) of the resulting supernatant was mixed with 1 mL of 0.5% (w/v) thiobarbituric acid (TBA) prepared in 20% (w/v) TCA. The reaction mixture was heated at 100 °C for 30 min and then immediately cooled in an ice bath to terminate the reaction. Absorbance was subsequently measured at 532 and 600 nm using a UV–Vis spectrophotometer. MDA concentration was calculated from the difference in absorbance between 532 and 600 nm using an extinction coefficient of 155 mM^{-1} cm^{-1} and expressed as nmol g^{-1} FW.

Antioxidant Enzyme Extraction and Assays

Enzyme extraction was performed according to Larkindale & Huang (2004). Briefly, 250 mg of fresh leaf tissue was homogenized in an ice-cold mortar with 100 mM potassium phosphate buffer (pH 7.0) containing 1% (w/v) polyvinylpyrrolidone (PVP) and 1 mM EDTA. The homogenate was centrifuged at 10,000 × g for 15 min at 4 °C, and the resulting supernatant was collected for subsequent enzyme assays. Total soluble protein concentration was determined according to the Bradford (1976) method.

Catalase (CAT) and peroxidase (POD) activities were determined following Elavarthi & Martin (2010). For CAT activity, the reaction mixture consisted of 2 mL of 50 mM sodium phosphate buffer (pH 7.0), 0.5 mL of 40 mM H_2O_2, and 0.5 mL of enzyme extract. CAT activity was determined by monitoring the decrease in absorbance at 240 nm resulting from H_2O_2 decomposition. For POD activity, 0.5 mL of enzyme extract was added to 2 mL of substrate solution containing 100 mM sodium phosphate buffer (pH 6.4) and 8 mM guaiacol, followed by incubation at 30 °C for 5 min. Subsequently, 1 mL of 24 mM H_2O_2 was added to initiate the reaction, and the increase in absorbance at 460 nm was recorded at 30-s intervals for 120 s. CAT and POD activities were expressed as U mg^{-1} protein.

Superoxide dismutase (SOD) activity was determined based on the inhibition of nitroblue tetrazolium (NBT) photoreduction according to Giannopolitis & Ries (1977). The reaction mixture contained 50 μL of enzyme extract, 195 μL of 0.1 M potassium phosphate buffer (pH 7.5), 1.2 mM Na_2EDTA, 150 mM methionine, 840 μM NBT, and 24 μM riboflavin. The reaction was initiated by illumination. A non-illuminated reaction mixture served as the blank, whereas an illuminated mixture lacking enzyme extract was used as the control. Absorbance was measured at 560 nm, and one unit of SOD activity was defined as the amount of enzyme causing 50% inhibition of NBT reduction per minute. Results were expressed as U mg^{-1} protein.

Ascorbate peroxidase (APX) activity was measured according to Nakano & Asada (1987). The reaction mixture (1.0 mL) contained enzyme extract, 50 mM phosphate buffer (pH 7.0), 0.5 mM ascorbic acid, 0.1 mM H_2O_2, and 0.1 mM EDTA. APX activity was determined by monitoring the decrease in absorbance at 290 nm due to ascorbate oxidation, using an extinction coefficient of 2.8 mM^{-1} cm^{-1}. One unit of APX activity was defined as the amount of enzyme catalyzing the oxidation of 1 μmol of substrate per minute at 25 °C, and the specific activity was expressed as U mg^{-1} protein.

Statistical analysis

All experiments were performed with three biological replicates. Data are presented as the mean ± standard deviation (SD). Prior to statistical analysis, the normality of the data distribution was assessed. Treatment effects were evaluated using two-way analysis of variance (ANOVA), and mean comparisons were performed using Duncan's multiple range test whenever significant differences were detected. Differences were considered statistically significant at $p \leq 0.05$. All

statistical analyses were conducted using SPSS software (V. 22.0).

Results

Photosynthetic and respiration rate

Heat stress markedly reduced the net photosynthetic rate (P_N), indicating a substantial impairment of photosynthetic performance under high-temperature conditions (Table 2). Compared with the control, heat-stressed plants exhibited a 47.07% reduction in P_N. In contrast, plants treated with SNP alone showed only a slight, non-significant decrease ($p \leq 0.05$), indicating that SNP did not adversely affect photosynthetic activity under normal conditions. Notably, SNP pre-treatment prior to heat exposure (Heat + SNP) significantly alleviated the inhibitory effects of heat stress. The decline in P_N was limited to 20.13% relative to the control, representing a 50.9% improvement compared with the heat stress treatment. Heat stress significantly enhanced dark respiration rate (R_d), reflecting increased respiratory metabolism under stressful conditions (Table 2). The R_d value increased by 114.29% under heat stress conditions. SNP treatment effectively attenuated this heat-induced rise in respiratory rate. Compared with the heat stress group, the Heat + SNP treatment reduced R_d by 31.11%, although respiration remained 47.62% higher than the control. These findings indicate that SNP partially restored respiratory homeostasis under heat stress. A similar trend was observed for the gross photosynthetic rate (P_G). Heat stress significantly ($p \leq 0.05$) reduced P_G by 27.71% compared with the control (Table 2). However, SNP application considerably improved photosynthetic capacity under heat stress, resulting in a 21.74% increase relative to the heat stress treatment. Consequently, the Heat + SNP plants exhibited only a 12.10% reduction compared with the control, demonstrating that SNP effectively preserved gross photosynthetic performance under elevated temperatures.

Table 2. Photosynthetic and respiration rates of tomato plants under control, heat stress, SNP, and heat + SNP treatments. Values represent the mean of three independent replications. Different letters indicate significant differences ($p \leq 0.05$).

Treatment	Net photosynthetic rate (P_N) μmol O_2 m^{-2} s^{-1}	Dark respiration rate (R_d) μmol O_2 m^{-2} s^{-1}	Gross photosynthetic rate (P_G) μmol O_2 m^{-2} s^{-1}
Control	15.19 ± 0.88[a]	2.10± 0.12[c]	17.29 ± 0.50[a]
Heat Stress	8.15± 0.32[c]	4.50± 0.25[a]	12.65± 0.40[c]
SNP	14.80± 0.51[a]	2.25 ± 0.15[c]	17.05± 0.55[a]
Heat +SNP	12.30± 0.41[b]	3.10± 0.20[b]	15.40± 0.45[b]

Chlorophyll *a* fluorescence transient (OJIP) and OEC activity (K-Band)

The chlorophyll *a* fluorescence transient (OJIP) and its normalized plot at the O and J phases are illustrated in Figure 1. As shown in Figure 1a, the chlorophyll *a* fluorescence levels vary across the different treatments. The kinetics of the K-band, reflecting the functional status of the OEC, exhibited variations under the experimental conditions (Figure 1 b). The control samples maintained stable kinetics with ΔW_{OJ} values near zero, indicating a fully functional OEC and balanced electron transport. Under heat stress, a pronounced K-band peak appeared rapidly, reaching a maximum amplitude of approximately 0.13 at 0.5 ms. This transient rise is a mark of OEC dissociation and the subsequent donor-side limitation of PSII. Conversely, plants treated with SNP alone showed kinetics comparable to the control. In the Heat + SNP treatment, the characteristic rise of the K-band was effectively suppressed; the amplitude was restricted to a peak of only 0.02–0.03 between 0.3 and 0.8 ms. This substantial mitigation suggests that SNP prevents the heat-induced structural disintegration of the OEC, thereby maintaining the efficiency of the OEC under thermal challenge.

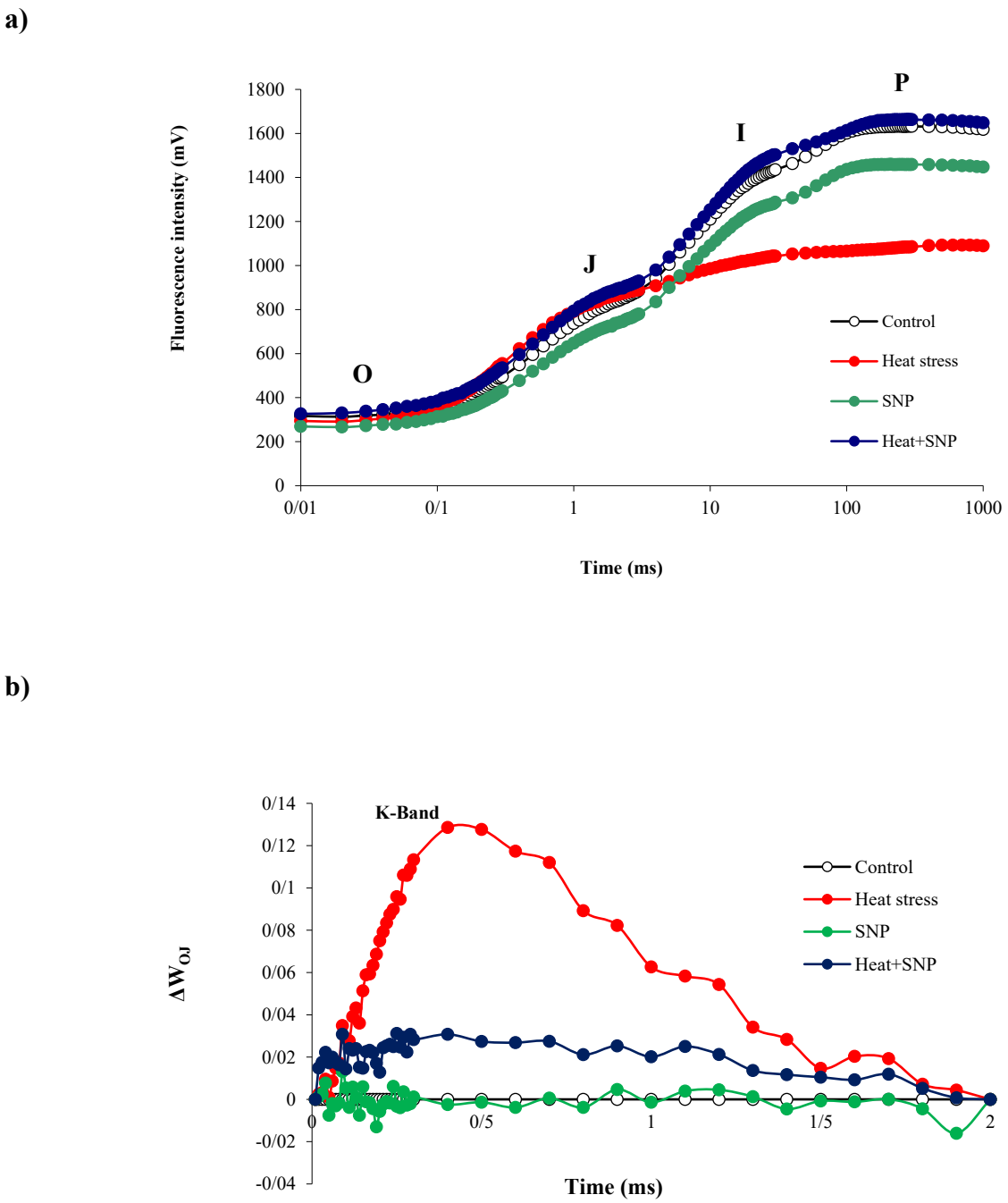
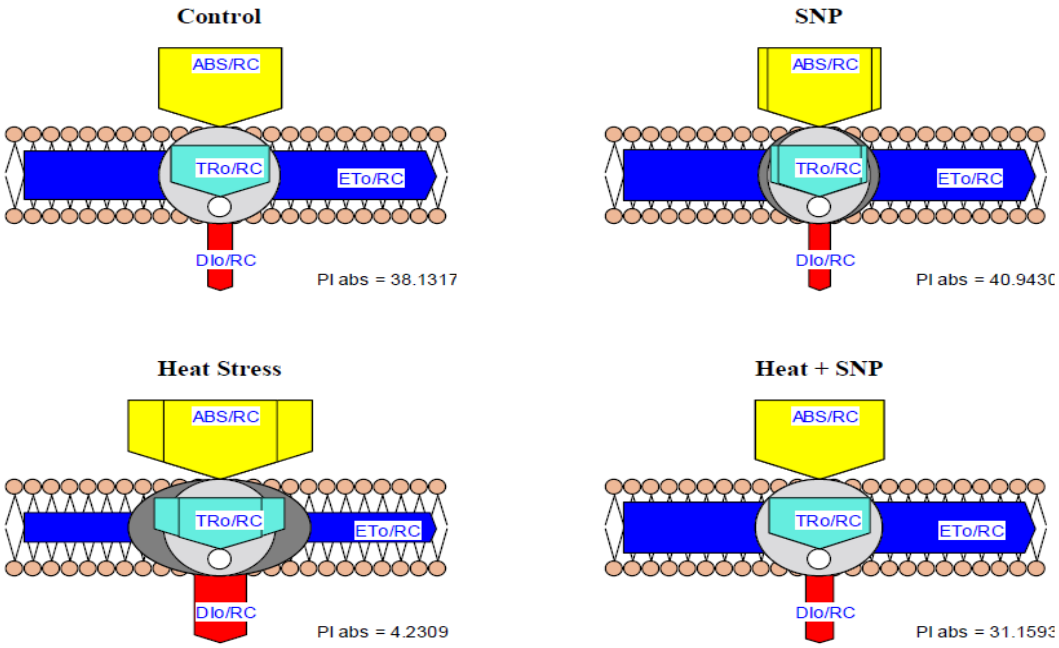


Figure 1. Chlorophyll *a* fluorescence transients (OJIP) (a) and appearance of the K-band as an indicator of Oxygen-Evolving Complex (OEC) disruption (b) in Photosystem II of tomato plants under control, heat stress, SNP, and Heat + SNP treatments.

JIP Parameters

Statistical analysis revealed that heat stress exerted detrimental effects on all biophysical and functional parameters of PSII (Figure 2). The performance index (PI_{ABS}) in the control and SNP treatments showed no statistically significant difference($p \leq 0.05$); however, under heat stress, this index decreased by 88.9%, representing a highly significant reduction compared to the control ($p \leq 0.05$). Nevertheless, the combination treatment (Heat + SNP) significantly mitigated this decline. Regarding energy dissipation (DIo), heat stress induced a 115.1% increase compared to the control, whereas the application of SNP under stress significantly reduced this dissipation ($p \leq 0.05$). Conversely, electron transport efficiency (ETo/RC) decreased by 34.2% under heat stress, but was fully recovered in the Heat + SNP treatment, showing no significant difference from the control ($p \leq 0.05$). Furthermore, heat stress

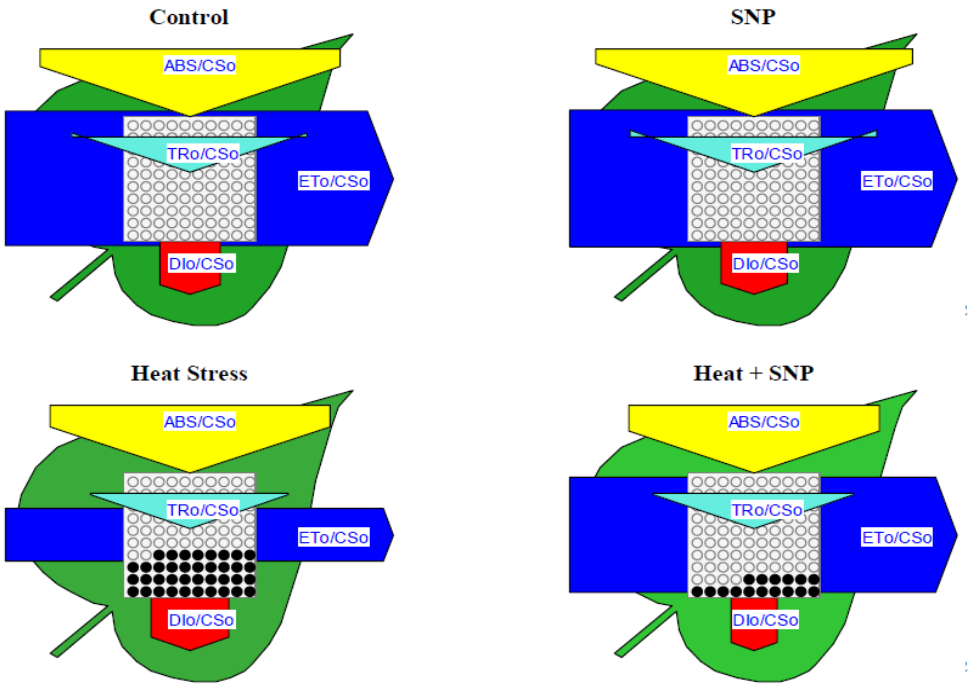
caused a significant increase in TRo/RC and ABS/RC by 35.4% and 51.8%, respectively, compared to the control ($p \leq 0.05$). In the Heat + SNP treatment, both parameters were significantly reduced compared to the heat-only stress. While heat stress led to a severe reduction in PI_{ABS}, the application of SNP significantly ameliorated this reduction($p \leq 0.05$), maintaining the performance index at a level close to the control



Treatment	ABS/RC	TRo/RC	ETo/RC	DIo/RC	PI_{ABS}
Control	1.62^{c}	1.30^{c}	0.79^{a}	0.33^{c}	38.13^{a}
SNP	1.63^{c}	1.32^{c}	0.81^{a}	0.31^{c}	40.94^{a}
Heat Stress	2.46^{a}	1.76^{a}	0.52^{b}	0.71^{a}	4.23^{c}
Heat +SNP	1.73^{b}	1.36^{b}	0.80^{a}	0.36^{b}	31.16^{b}

Figure 2. Flux parameters per active reaction center (ABS/RC, TRo/RC, ETo/RC, DIo/RC and PI_{ABS}) in tomato plants under control, heat stress, SNP, and heat + SNP treatments. Values represent the mean of three independent replications. Different letters indicate significant differences ($p \leq 0.05$).

The assessment of photosynthetic efficiency using the phenomenological yield model revealed significant alterations in the functional capacity of the photosynthetic apparatus under thermal stress (Figure 3). Heat stress caused a dramatic decline in the functional capacity of reaction centers, with RC/CS decreasing by 55.9% compared to the control ($p \leq 0.05$). This was accompanied by a sharp reduction in electron transport efficiency (ETo/CS), which dropped by 68.5%, and a significant decrease in the overall energy processing capacity (ABS/CS and TRo/CS), which fell by 33.1% and 37.9%, respectively ($p \leq 0.05$). Conversely, the dissipation of energy relative to the cross-section (DIo/CS) increased by 22.6% under heat stress. The application of SNP significantly mitigated these declines ($p \leq 0.05$). In the Heat + SNP treatment, RC/CS was maintained (10.9% reduction from control compared to the 55.9% loss in heat-only stress). Similarly, the efficiency of electron transport (ETo/CS) was partially restored and significantly outperformed the heat-stressed plants ($p \leq 0.05$). The DIo/CS in the Heat + SNP group was remarkably close to the control levels, indicating that SNP effectively regulated the ratio of energy dissipation to the functional cross-section.



Treatment	ABS/CS	TRo/CS	ETo/CS	DIo/CS	RC/CS
Control	1632^{b}	1336^{b}	797^{a}	296^{c}	1002^{a}
SNP	1664^{a}	1349^{a}	776^{b}	305^{c}	960^{b}
Heat Stress	1092^{d}	829^{d}	229^{d}	363^{a}	443^{d}
Heat +SNP	1459^{c}	1209^{c}	723^{c}	320^{b}	892^{c}

Figure 3. Phenomenological fluorescence parameters (ABS/CS, TRo/CS, ETo/CS, DIo/CS, and RC/CS) of tomato plants under control, heat stress, SNP, and heat + SNP treatments. Values represent the mean of three independent replications. Different letters indicate significant differences ($p \leq 0.05$).

Heat stress significantly altered the photochemical (k_P) and non-photochemical (k_N) de-excitation rate constants (Table 3). Compared with the control, heat stress reduced k_P by 19.31%, from 2.59 to 2.09, whereas SNP treatment alone increased k_P by 11.97%, reaching the highest value (2.90). Pre-treatment with SNP partially alleviated the inhibitory effect of heat stress, increasing k_P by 16.75% compared with heat-stressed plants, although the value remained 5.79% lower than that of the control ($p \leq 0.05$). Conversely, heat stress increased k_N by 50.82% compared with the control (0.92 *vs*. 0.61), indicating enhanced non-photochemical energy dissipation. SNP treatment alone did not significantly affect k_N (0.60). However, SNP pre-treatment significantly reduced k_N by 16.30% relative to heat-stressed plants, although the value remained 26.23% higher than that of the control ($p \leq 0.05$).

Table 3. Photochemical (k_P) and non-photochemical (k_N) de-excitation rate constants of tomato plants under control, heat stress, SNP, and heat + SNP treatments. Values represent the mean of three independent replications. Different letters indicate significant differences ($p \leq 0.05$).

Parameter (a. u.)	Treatment			
	Control	SNP	Heat	Heat + SNP
k_P	2.59^{b}	2.90^{a}	2.09^{d}	2.44^{c}
k_N	0.61^{c}	0.60^{c}	0.92^{a}	0.77^{b}

Oxidative stress markers

The accumulation of H_2O_2in tomato varied significantly ($p \leq 0.05$) among treatments (Figure 4). Compared with the control, SNP alone reduced H_2O_2 content by 14.35%. In contrast, heat stress significantly ($p \leq 0.05$) increased H_2O_2 by 58.44%, relative to the control. However, the combined Heat + SNP treatment lowered H_2O_2 by 17.84% compared with the heat-alone treatment. This observation indicates that heat exposure strongly enhanced reactive oxygen species generation, while SNP alleviated oxidative damage. The reduction in H_2O_2under SNP suggests that nitric oxide released from SNP may have activated antioxidant defense systems and/or directly scavenged ROS, limiting oxidative damage. The partial recovery under Heat + SNP indicates that SNP mitigated heat-induced oxidative stress.

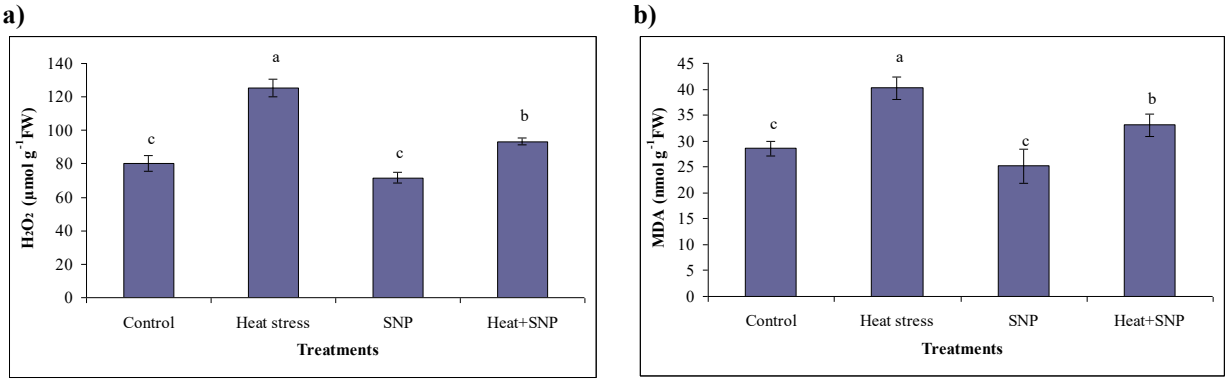


Figure 4. Changes in the levels of oxidative markers, including H_2O_2 (a) and MDA (b), in tomato plants under control, heat stress, SNP, and heat + SNP treatments. Values represent the mean of three independent replications. Different letters indicate significant differences ($p \leq 0.05$).

Antioxidant enzymes activity

The application of SNP as an exogenous NO donor significantly ($p \leq 0.05$) modulated the antioxidant defense system in tomato under heat stress (Figure 5). Exposure to heat stress alone resulted in a significant ($p \leq 0.05$) reduction in the activities of key antioxidant enzymes compared to the control (CAT 36.7%, POD 43.7%, SOD 28.4% and APX 38.1%). However, the application of SNP under heat stress (Heat + SNP) effectively reversed this inhibition. Compared to the heat-stressed plants, the Heat + SNP treatment showed significant increases in the activity of CAT 112.5%, POD 161.2%, SOD 73.5%, and APX 89.2%, highlighting the important role of SNP in mitigating heat-induced oxidative toxicity.

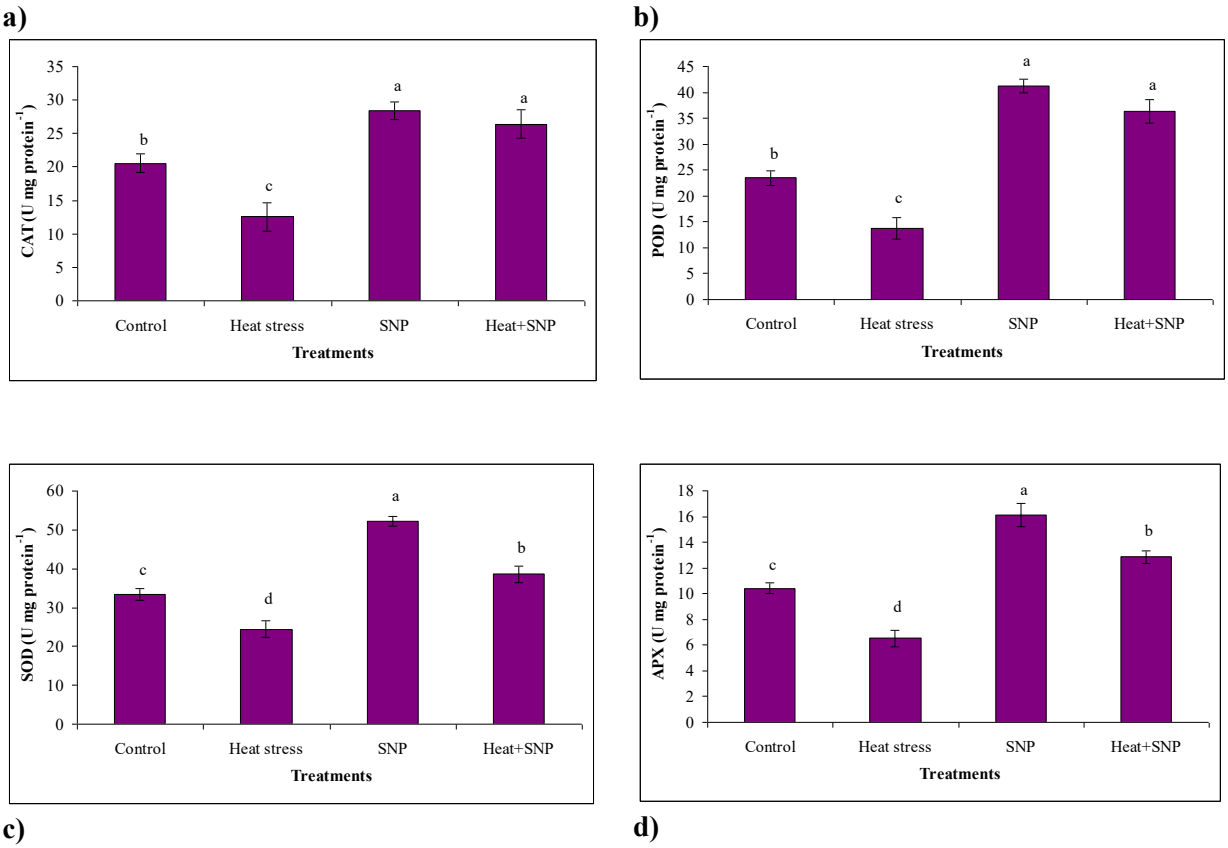


Figure 5. Activities of antioxidant enzymes, including CAT (a), POD (b), SOD (c), and APX (d) (U mg protein^{-1}), in tomato plants subjected to control, heat stress, SNP, and heat + SNP treatments. Values are means of three independent replicates. Different letters indicate significant differences ($p \leq 0.05$).

Discussion

Photosynthetic and respiratory rates

Heat stress is widely recognized as one of the most detrimental abiotic stresses affecting plant growth because it disrupts photosynthesis while simultaneously increasing respiratory carbon losses (Zahra et al., 2023). The present study clearly demonstrated that elevated temperature significantly reduced both net and gross photosynthetic rates while markedly stimulating dark respiration. These physiological responses indicate that heat stress severely disturbs plant carbon metabolism by decreasing carbon assimilation and increasing carbon consumption. The 47.07% reduction in P_N under heat stress observed in the present study is consistent with the well-established effects of heat stress on the photosynthetic apparatus. High temperatures destabilize thylakoid membranes, reduce chlorophyll stability, inhibit the activity of PSII, and decrease the catalytic efficiency of Rubisco (Allakhverdiev et al., 2008; Billah et al., 2024; Zahra et al., 2023). Moreover, heat-induced stomatal closure limits CO_2 diffusion into the leaves, while oxidative stress generated by excessive ROS further damages chloroplast structures and photosynthetic proteins (Zahra et al., 2023). Collectively, these factors lead to a pronounced decline in photosynthetic carbon fixation. The increase of 114.29% in dark respiration indicates that plants require substantially more metabolic energy under heat stress. Enhanced respiration under elevated temperatures is generally associated with increased ATP demand for protein turnover, membrane repair, antioxidant metabolism, and maintenance of cellular homeostasis (Scafaro et al., 2021). Although respiratory activation provides energy for stress adaptation, excessive respiration also accelerates carbohydrate depletion, thereby reducing biomass accumulation and crop productivity (Scafaro et al., 2021). Pre-treatment with SNP significantly alleviated the adverse effects of heat stress by simultaneously improving photosynthetic performance and suppressing excessive respiratory activity. SNP treatment

markedly improved the overall carbon balance under heat stress, as evidenced by the higher P_N and P_G values together with the lower R_d compared with heat-stressed plants. The protective effects observed following SNP application are likely associated with nitric oxide released from SNP, although the specific contribution of NO requires further confirmation using appropriate NO scavengers. Several mechanisms have been proposed to explain the protective effects of SNP under heat stress (Naaz et al., 2025). Previous studies have shown that SNP treatment enhances the activities of antioxidant enzymes, including SOD, CAT, APX, and GR, thereby reducing ROS accumulation and protecting chloroplast membranes from oxidative damage (Wani et al., 2021). In addition, SNP application has been reported to maintain chlorophyll content, preserve PSII functionality, sustain Rubisco activity, and improve stomatal regulation, all of which contribute to enhanced photosynthetic performance under heat stress (Lopes-Oliveira et al., 2021). SNP treatment has also been associated with improved mitochondrial function and reduced respiratory carbon loss through better maintenance of cellular redox homeostasis and metabolic efficiency (Scafaro et al., 2021).

The present findings are consistent with previous studies demonstrating the beneficial effects of SNP application under heat stress. Dehbozorgi et al. (2025) reported that SNP enhanced photosynthetic gas exchange and antioxidant capacity in tomato exposed to elevated temperatures. Similarly, Gautam et al. (2022) observed that SNP improved PSII efficiency and chlorophyll retention in rice, whereas Siddiqui et al. (2017) showed that SNP maintained Rubisco activity and carbon assimilation in wheat under heat stress. Hasanuzzaman et al. (2012) also demonstrated that SNP application enhanced photosynthetic performance and antioxidant defense through improved ROS detoxification, while Fancy et al. (2017) highlighted the importance of nitric oxide signaling in regulating plant adaptation to environmental stresses and maintaining cellular homeostasis. These studies collectively support the improvements in P_N, P_G, and Rd observed in the present investigation. Overall, the improved carbon balance observed following SNP treatment appears to result from the combined maintenance of photosynthetic performance, reduction of oxidative damage, and limitation of excessive respiratory carbon loss. Together with the chlorophyll fluorescence and antioxidant data presented in this study, these findings suggest that SNP treatment contributes to enhanced thermotolerance by maintaining photosynthetic functionality under heat stress rather than providing direct evidence of structural protection of the photosynthetic apparatus.

OEC activity (K-Band)

The appearance of the K-band at the 0.3 ms transient is widely regarded as an indicator of changes in the functional status of the OEC on the donor side of PSII. Our results clearly demonstrate that high temperature impaired OEC functionality, as evidenced by the sharp increase in ΔW_{OJ} in heat-stressed plants. This phenomenon is consistent with previous studies showing that heat stress disrupts electron donation from the donor side of PSII and is frequently associated with functional impairment of the OEC (Arslan, 2023; Chen et al., 2017; Mihaljević et al., 2024). The suppression of the K-band following SNP treatment suggests that SNP contributed to maintaining OEC functionality and donor-side electron transport under heat stress. Our observation of K-band formation under thermal stress agrees with the findings of Khalilpoor & Jafarinia (2017) in oats and Mirzaei Chegeni et al. (2024) in tomato, which identified the K-band as a sensitive indicator of OEC dysfunction under abiotic stress conditions. Likewise, Dehbozorgi et al. (2025) reported that abiotic stress induced similar alterations in donor-side electron transport in wheat, indicating that disruption of OEC functionality is a common response to environmental stress across plant species. Although previous studies have primarily attributed the beneficial effects of SNP to enhanced antioxidant capacity, improved photosynthetic performance, and protection of the photosynthetic apparatus, relatively few investigations have evaluated its influence on OEC functionality using K-band analysis in combination with detailed OJIP parameters. In the present study, the integration of K-band analysis with complementary chlorophyll

fluorescence and physiological measurements provides additional evidence that SNP treatment contributed to maintaining PSII performance under heat stress. Previous reports have suggested that severe heat stress may cause irreversible damage to the donor side of PSII (Čajánek et al., 1998; Mathur et al., 2011).

In contrast, the significant reduction in the K-band observed following SNP treatment indicates that SNP alleviated heat-induced impairment of OEC functionality. Nevertheless, the present findings should be interpreted as physiological evidence of improved OEC performance rather than direct evidence of structural stabilization of the manganese cluster or OEC proteins. Confirmation of structural protection would require direct analyses of OEC components, such as the PsbO, PsbP, and PsbQ proteins or the Mn_4CaO_5 cluster, which were beyond the scope of the present study.

JIP Parameters

The drastic reduction in PI_{ABS} under heat stress indicates extensive damage to membrane structures and disruption of photochemical processes within PSII. This 88.9% decrease suggests that thermal stress targets not only energy transfer efficiency but also the overall capacity of the photosynthetic apparatus. The substantial increase in DI_0 under stress (115.1%) indicates enhanced dissipation of absorbed excitation energy, which reflects the activation of protective energy dissipation mechanisms under heat stress. This interpretation is further supported by the significant increase in the non-photochemical de-excitation rate constant (k_N) observed in the present study, indicating that a larger proportion of absorbed energy was dissipated through non-photochemical pathways. Conversely, the decline in the photochemical de-excitation rate constant (k_P) demonstrates reduced utilization of excitation energy for photochemistry. These findings are consistent with previous studies showing that severe environmental stresses increase energy dissipation to minimize photooxidative damage (Sharma et al., 2023; Zuo, 2025). The significant recovery of ET_0/RC following SNP treatment suggests that SNP contributed to maintaining electron transport efficiency under heat stress, thereby alleviating the impairment of electron flow commonly associated with thermal stress (Fancy et al., 2017; Mirzaei Chegeni et al., 2024).

Furthermore, the significant increases in TR_0/RC and ABS/RC under heat stress reflect greater excitation pressure on the remaining active reaction centers, as partial inactivation of PSII results in a higher energy load per functional center (Shahsavandi et al., 2024). The partial restoration of these parameters following SNP treatment is consistent with the observed improvements in antioxidant enzyme activities and photosynthetic performance, suggesting more efficient energy utilization and reduced excitation pressure on PSII. Rather than indicating direct structural protection, these physiological responses suggest improved maintenance of PSII functionality under heat stress. Overall, the results demonstrate that although heat stress caused a severe decline in PI_{ABS}, SNP treatment substantially improved photosynthetic performance by promoting more efficient energy partitioning and maintaining electron transport. The results obtained from the phenomenological model demonstrate that heat stress fundamentally impaired the functional efficiency of the photosynthetic apparatus by reducing the number of active reaction centers per cross-section (RC/CS) and disrupting electron transport (ET_0/CS). The 68.5% reduction in ET_0/CS under heat stress indicates a marked decline in the capacity of PSII to convert absorbed light energy into photochemical energy, which is consistent with functional impairment of the donor and acceptor sides of PSII. This observation agrees with the findings of Chen et al. (2017), who reported that elevated temperatures disrupt the coupling between light harvesting and electron transport, thereby reducing photosynthetic efficiency. The 22.6% increase in DI_0/CS indicates that a larger fraction of the absorbed energy was dissipated rather than utilized for photochemistry, which is in agreement with the increased k_N values observed in the present study. Such responses are characteristic of plants exposed to thermal stress and serve to reduce excess excitation pressure on PSII (Mihaljević et al., 2024). Application of SNP partially restored RC/CS and ET_0/CS while reducing DI_0/CS, indicating improved functional

performance of PSII under heat stress. These changes, together with the increases in k_P and decreases in k_N, demonstrate that SNP treatment promoted a more balanced partitioning of absorbed excitation energy between photochemical utilization and non-photochemical dissipation. These findings are also consistent with previous studies reporting that SNP treatment enhances antioxidant capacity and preserves photosynthetic performance under abiotic stress conditions (Mirzaei Chegeni et al., 2024; Dehbozorgi et al., 2025). However, the present results should be interpreted as evidence of improved PSII functionality rather than direct structural stabilization of the photosynthetic apparatus.

Oxidative stress markers

The present findings are consistent with numerous reports showing that abiotic stresses increase H_2O_2 and MDA levels in plants due to excessive ROS production and oxidative membrane damage (Borjian et al., 2025; Kalami et al., 2025). Similar increases in ROS accumulation and lipid peroxidation under heat stress have been reported in several species, including rice (*Oryza sativa*), tomato (*Solanum lycopersicum*), and wheat (*Triticum aestivum*) (Buttar et al., 2020; Liu et al., 2025; Zhuo et al., 2019). These studies attribute the accumulation of H_2O_2 and MDA to disruption of the photosynthetic and respiratory electron transport chains, reduced antioxidant capacity, and enhanced oxidative damage under abiotic stress conditions. The reduction in H_2O_2 and MDA observed following SNP treatment in the present study agrees with previous reports demonstrating that SNP application alleviates oxidative damage under salinity, drought, chilling, and heat stress by enhancing antioxidant defense systems and improving cellular redox homeostasis (Dehbozorgi et al., 2025; Mirzaei Chegeni et al., 2024; Silveira et al., 2017). Similarly, Dehbozorgi et al. (2025) and Mirzaei Chegeni et al. (2024) reported that SNP treatment significantly reduced oxidative damage in wheat and tomato, respectively, whereas Silveira et al. (2017) demonstrated that SNP alleviated lipid peroxidation and ROS accumulation under abiotic stress conditions. Nevertheless, the inability of SNP treatment to restore H_2O_2 and MDA completely to control levels indicates that its protective effect was partial under the experimental conditions of the present study. This observation is consistent with previous reports suggesting that the effectiveness of SNP treatment depends on factors such as stress severity, plant species, developmental stage, and SNP concentration (Dehbozorgi et al., 2025; Farahani et al., 2025; Mirzaei Chegeni et al., 2024; Silveira et al., 2017).

Antioxidant enzymes activity

Our results demonstrate that heat stress impairs the antioxidant defense system in tomato, as evidenced by the sharp decline in CAT, POD, SOD, and APX activities. This inhibition is likely associated with temperature-induced disruption of enzyme conformation and catalytic activity, resulting in reduced ROS-scavenging capacity (Buttar et al., 2020; Liu et al., 2025; Zhuo et al., 2019). SNP treatment significantly alleviated these adverse effects by markedly enhancing the activities of the antioxidant enzymes (Wani et al., 2021). The substantial recovery of antioxidant enzyme activities following SNP treatment is consistent with previous studies reporting that SNP application enhances antioxidant defense and improves cellular redox homeostasis under abiotic stress conditions (Ma et al., 2022; Wani et al., 2021). Although the protective responses observed in the present study are likely associated with nitric oxide released from SNP, confirmation of the specific role of NO would require additional experiments using NO scavengers. The restoration of SOD activity, which constitutes the first enzymatic line of defense against oxidative stress, likely promoted the rapid conversion of superoxide radicals to H_2O_2, whereas the enhanced activities of CAT, POD, and APX facilitated the subsequent detoxification of H_2O_2. Together, these coordinated antioxidant responses probably contributed to limiting oxidative damage and maintaining photosynthetic performance under heat stress. These observations are consistent with the improvements in chlorophyll fluorescence parameters, photosynthetic gas exchange, and K-band responses observed in the present study,

suggesting that enhanced antioxidant capacity contributed to the maintenance of PSII and OEC functionality under heat stress. However, these findings should be interpreted as physiological evidence of improved functionality rather than direct evidence of structural protection of the OEC. Overall, the present results demonstrate that SNP treatment strengthened the antioxidant defense system and enhanced the physiological tolerance of tomato plants to heat stress by improving redox balance and reducing oxidative damage.

Conclusion

This study demonstrates the beneficial effects of SNP treatment in mitigating heat stress-induced damage in tomato plants, with particular emphasis on maintaining photosynthetic performance and OEC functionality. Our findings reveal a close relationship between biochemical defense and photosynthetic performance under heat stress. At the metabolic level, heat stress severely compromised carbon assimilation by drastically reducing the net photosynthetic rate and increasing dark respiration, leading to an overall imbalance in carbon metabolism. However, SNP pre-treatment effectively mitigated these adverse effects, maintaining a more favorable carbon balance. This physiological improvement was accompanied by enhanced antioxidant defense. While heat stress suppressed the activities of key antioxidant enzymes (CAT, POD, SOD, and APX) and increased oxidative stress, as indicated by elevated H_2O_2 and MDA contents, SNP treatment markedly enhanced antioxidant enzyme activities and reduced oxidative damage. Chlorophyll fluorescence analyses further demonstrated that heat stress impaired PSII performance, as evidenced by the marked reduction in PI_{ABS} and the appearance of the K-band. The suppression of the K-band following SNP treatment suggests improved maintenance of OEC functionality and donor-side electron transport under heat stress. In combination with the improvements observed in OJIP parameters, photosynthetic gas exchange, and antioxidant capacity, these findings indicate that SNP treatment contributed to preserving the functional performance of the photosynthetic apparatus. However, the present results provide physiological evidence of improved OEC functionality rather than direct evidence of structural stabilization of the OEC or the Mn_4CaO_5 cluster. Overall, SNP treatment enhanced heat tolerance in tomato plants by improving antioxidant defense, maintaining photosynthetic performance, optimizing energy utilization, and reducing oxidative damage. Future studies incorporating nitric oxide scavengers and direct analyses of OEC components will be required to verify the specific role of nitric oxide and the structural basis of the protective responses observed in the present study.

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Authors' Contributions

Fatemeh Dehbozorgi: Data curation, Funding acquisition, Investigation, Research, Resources, Visualization, Writing–original draft. Mojtaba Jafarinia: Conceptualization, Formal analysis, Project administration, Methodology, Supervision, Writing– review and editing, Validation. Ali Akbar Ghotbi-Ravandi: Software, Writing – review and editing.

Availability of Data and Materials

The data that support the findings of this study are available from the corresponding authors, upon reasonable request.

Conflict of Interests

The author declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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