



Role of Sirtuins in Heat Stress Tolerance under Elevated CO₂ in *Arabidopsis*: Photosynthesis, Antioxidant System and Redox Regulation

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Abstract

Current knowledge about what the protective roles of elevated CO₂ concentration (eCO₂) on chlorophyll fluorescence, PSII photochemistry and antioxidant capacity of *Arabidopsis* with heat stress remains insufficient. Besides, there is a lack of information on the tolerance mechanism of plants against stress in the presence or absence of sirtuin genes. This study aimed to investigate how eCO₂ (800 ppm) mitigated heat stress (36 °C)-dependent oxidative damages through analyzing growth, water content, gas exchange, chlorophyll fluorescence, electron flux efficiency in PSII, antioxidant capacity and lipid peroxidation in *Arabidopsis thaliana* cv. Columbia-0 (Col-0) and its sirtuin-deficient mutant (*srt2*) plants. The focus was on investigating the function of the SRT2 gene, particularly in comparison with wild-type plants. Therefore, the sirtuin inhibitor (sirtinol, 1 µM S) was applied to Col-0 plants and the effects of ambient (400 ppm) and eCO₂ heat (H) under stress were also examined. The reductions in growth and water content of heat stress-treated Col-0 and *srt2* mutants were eliminated by eCO₂ exposure. The elevated CO₂ was removed the reductions on chlorophyll fluorescence, carbon assimilation rate and carboxylation efficiency dependent by H stress in Col-0 plants, but not in *srt2* mutants. S supplementation to Col-0 plants produced effects similar to those observed under stress across all measured parameters. While the structural indicators and electron fluxes of PSII and performance indices were disrupted by H stress, eCO₂ provided the positive effects on the photochemistry of PSII in the wild type of *Arabidopsis*. Interestingly, the eCO₂-triggered alleviation against stress in *srt2* mutants was not maintain. The induced (SOD and GR) and reduced activities (POX, APX, AsA/DHA and GSH redox state) of antioxidant systems point to increased H₂O₂ accumulation in both Col-0 (123%) and *srt2* mutant (40%). Both of the *Arabidopsis* plants experienced oxidative stress as proved by the high levels of lipid peroxidation. eCO₂ under H stress resulted in remarkable decline in H₂O₂ accumulation, which contributed to POX and AsA regeneration in Col-0 and POX, AsA and GSH redox status in *srt2*. Sirtinol application to Col-0 plants also eliminated eCO₂-mediated protection on TBARS content. This study provides the first evidence of SRT2 involvement in CO₂-mediated heat stress tolerance.

Keywords *Arabidopsis* · Elevated CO₂ · Heat stress · Sirtuin · Sirtinol · *srt2* mutant

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Introduction

Histone deacetylases (HDACs) as chromatin regulators regulate chromatin structure, metabolic activities, translation and transcription by deacetylation of non-histone proteins (Cui et al. 2023). Besides, HDACs-mediated deacetylation of lysine residues in histones is essential for gene regulation under stress. HDACs are classified as three types: (i) zinc-dependent the Reduced Potassium Deficiency3 (RPD3)-like family, (ii) plant-specific HDAC (HD-tuin) family and (iii) NAD⁺-dependent Silent Information Regulator2 (SIR2)-like (sirtuin) family (Ueda et al. 2017). The previous reports showed that there are negative/positive regulation of the genes responsible for HDACs against stress conditions. For example, HDA9 (class I, RPD3-like HDAC) inhibited the DNA binding activity of WRKY53 that is a transcription factor rolling as activator of stress-responsive genes (Zheng et al. 2020). On the other hand, HDA6-mediated gene expression switch is associated with the defense responses in drought stressed-plants (Kim et al. 2017). Much needs to be investigated about the roles of HDACs on the adaptive mechanisms between the deficient mutants and wild type plants under stress conditions.

Sirtuins are NAD⁺-dependent ubiquitous enzymes belonging to histone deacetylases (Bruscalupi et al. 2023) and are present in nucleus, mitochondria, chloroplast and cytosol (Chen et al. 2015). The findings about the sirtuins are generally focused on the importance of gene expression, DNA repair and, silencing in diabetes, obesity and, cancer and cell metabolism in mammals (Soccio et al. 2018). Although mammalian and yeast sirtuins have been extensively reported in last decades, the information on plant sirtuins remains limited. Plant sirtuins have role in gametogenesis, ripening of fruits, photosynthetic activity, removal of oxidative damage and auxin signaling (Cucurachi et al. 2012; Zhao et al. 2015). In *Arabidopsis*, the sirtuin-related genes SRT1 and SRT2 are both encoded in the nuclear genome, with SRT1 localized in the nucleus and SRT2 targeted to mitochondria. In contrast, the tomato SRT2 protein localizes mainly in the nucleus and cytoplasm (König et al. 2014; Zhao et al. 2015). It has been detected that AtSRT2 gene participate in the regulation of mitochondrial energy metabolism. This function of AtSRT2 is dependent on deacetylation of the proteins situated in inner membrane of mitochondria (ADP/ATP carriers and ATP synthase) (König et al. 2014). SRT1 and SRT2 (SRT2-1) are involved in epigenetic regulation of gene expression related to stress response, energy/carbon metabolism and redox homeostasis in *Arabidopsis*, strawberry and rice (Liu et al. 2017; Zhang et al. 2017). The various inhibitors such as sirtinol participate in the inhibition of SRT2 gene expression in *Arabidopsis* (Grozinger et al. 2001). After sirtinol

treatment (sirtuin deacetylase activity inhibitor), the nuclear abundance of OsGAPDH1, which is a typical glycolytic protein, enhanced in rice protoplasts (Zhang et al. 2017). It is reported that the over-expression in AtSRT1 causes a hypersensitivity to abscisic acid, salt and osmotic stress (Liu et al. 2017). A previous study reported that sirtuin-mediated regulatory proteins involved in defense system in response to stress (König et al. 2014). On the other hand, *srt2-1* and *srt2-2* mutants showed a low germination rate under control or salinity (Tang et al. 2022). Considering all data, in case absence/presence of SRT, the exact mechanisms of this gene on water regulation, photosynthesis and antioxidant activity are still largely unknown in plants under stress conditions.

As well as epigenetic regulation, the basic plant process such as glycolysis and tricarboxylic acid (TCA) cycle is also affected by abiotic stress factors. One of them is the changes on atmospheric CO₂ concentrations (aCO₂), currently at about 385 µmol mol⁻¹ (Wang et al. 2023). CO₂ concentrations in atmosphere could nearly reach 800 µmol mol⁻¹ as of 2100 (Zhang et al. 2023). The drastic elevated CO₂ levels (eCO₂) triggered by global climate changes alter photosystem efficiency, respiration and growth (Baluška and Mancuso 2020). The plants are exposed to heat stress together with eCO₂. The plants are exposed to heat stress together with eCO₂, since co-existence of different stress factors can activate more complex responses and defense pathways than single stresses (Zhang et al. 2019a). High temperature stress disrupts the stability and fluidity of membranes, water status, protein oxidation, pigment composition, excitation energy on PSII and enzyme activity in plants (Jagadish et al. 2021). The reason of damages induced by stress is accumulation of reactive oxygen species (ROS), which is scavenged through antioxidant defense system including superoxide dismutase (SOD), peroxidase (POX), catalase (CAT) and ascorbate peroxidase (APX). As a result of both stresses (heat and eCO₂), plants try to resist damage by inducing arrangement in stomata, photosynthesis and flowering time (Macabuhay et al. 2018). Interestingly, the recent meta-analyses showed that eCO₂ triggers biomass production of plants by regulating carbon fixation, NADPH formation as reducing agent, photorespiration and hormonal pools under stress (Byeon et al. 2021). Rising CO₂ levels alter the stomatal density and stomatal conductance and then can improve water use efficiency in the heat stressed-plants (Avila et al. 2020; Zinta et al. 2018). These changes suppress photorespiration and, excessive ROS production and enhance photosynthetic CO₂ exchange rate (Zhou et al. 2020). Selim et al. (2021) indicated a promotion in osmotic adjustment by accumulating proline, soluble sugars and glycine betaine in arsenic-treated maize and barley plants under eCO₂ conditions. The assessments on the interactions of eCO₂ with adverse conditions remain inconclusive in plants. Besides, there is a

significant gap about whether sirtuins are involved in defensive processes together with $e\text{CO}_2$ under extreme heat stress in plants. Especially, the combined effects of $e\text{CO}_2$ and heat stress on photosynthesis responses and antioxidant system need further study in SRT2-defective mutant plants by comparing wild type. To better determine the interaction of the SRT gene with elevated CO_2 under heat stress, sirtinol, an SRT inhibitor, to the wild type plants of *Arabidopsis* was also applied. The aimed of the current study was to evaluate the interaction between ambient/elevated CO_2 concentrations and sirtuins on water relations, PSII photochemistry, redox regulation and stress responses (ROS accumulation and antioxidant system) under heat stress in wild type Col-0 and *srt2* mutants, lacking the SRT2 gene of *Arabidopsis*.

Materials and Methods

Plant Material and Experimental Design

Arabidopsis thaliana ecotype Columbia-0 (Col-0) and SRT2 gene-deficient mutant (*srt2*, SALK_149295C) seeds were obtained from NASC (European Arabidopsis Stock Center in Nottingham). After the germination process, *Arabidopsis* seeds were transferred to Murashige and Skoog (MS) medium (Murashige and Skoog 1962) in petri plate for 21 d. Seedlings were grown in a controlled growth chamber under 400 ppm CO_2 concentration, 16/8 h light/dark regime at 24 °C, 70% relative humidity and $350 \mu\text{mol m}^{-2} \text{s}^{-1}$ photosynthetic photon flux density. Three-week-old *A. thaliana* seedlings were applied to heat stress, elevated CO_2 levels (800 ppm) and sirtinol application (1 μM S). For heat stress, it reached the target temperature (36 °C) by increasing 4 °C in 4 hours (h), and *A. thaliana* plants were exposed to 36 °C for 24 h. During harvesting, the samples were immediately stored in liquid nitrogen (-196 °C). Plants were harvested after 24 h of treatment and the leaves were stored at -86 °C until further analyses. During the homogenization stage of the analysis, the samples were first mechanically broken down in liquid nitrogen, and then homogenization buffer was added. The treated temperature levels were determined according to the previous studies reported by Rizhsky et al. (2004), Swindell et al. (2007) and Bevilacqua et al. (2019). *Arabidopsis* plants were exposed the elevated CO_2 concentrations (800 ppm) as reported by Wei et al. (2022) and Li et al. (2022). To further assess whether SRT2 gene were involved in defense mechanisms and stress responses, 1 μM sirtinol application, sirtuin inhibitor, was applied to wild type Col-0 of *Arabidopsis*.

Determination of Growth, Water Content and Proline Content

The relative growth rate (RGR) was calculated according to the procedure suggested by Hunt et al. (2002). After harvest, six leaves were obtained and their fresh weight (FW) was determined. The leaves were floated on de-ionised water for 6 h and the turgid tissue was blotted dry prior to determining turgid weight (TW). Dry weight (DW) was determined after oven drying at 70 °C. The leaf relative water content (RWC) was calculated by the following formula (Smart and Bingham 1974):

$$\text{RWC (\%)} = [(\text{FW}-\text{DW}) / (\text{TW}-\text{DW})] \times 100$$

Pro content was calculated according to Bates et al. (1973). The leaves were homogenized in 3% sulphosalicylic acid and homogenate was filtered through filter paper. After addition of acid ninhydrin and glacial acetic acid, the mixture was heated at 100 °C. The mixture was extracted with toluene and the absorbance of fraction with toluene aspired from liquid phase was measured at 520 nm.

Determination of Gas Exchange Parameters

A portable fluorometer (Handy PEA, Hansatech, King's Lynn, UK) was used to determine the maximal quantum yield of PSII photochemistry (F_v/F_m). Carbon assimilation rate (A), stomatal conductance (g_s), intercellular CO_2 concentration (C_i) and transpiration rate (E) were measured with a portable gas exchange system (LCpro+; ADC, Hoddesdon, UK) (Zhang et al. 2019b).

Determination of Chlorophyll Fluorescence and OJIP Analysis

The changes on the photochemistry of PSII were identified through Handy PEA+ (Hansatech). The calculations for the function of photosystems were used by Plant Efficiency Analyzer and the definition of the measured parameters was shown in Supplementary Table S1.

Determination of H_2O_2 Content and Lipid Peroxidation

Determination of H_2O_2 content was measured according to Liu et al. (2010). The samples were homogenized in cold acetone and centrifuged. The supernatant was mixed with titanium reagent and then ammonium hydroxide was added to precipitate the titanium-peroxide complex. The reaction mixture was centrifuged. The pellet was washed with cold acetone and dissolved. The absorbance of the solution was

measured at 410 nm. H_2O_2 concentrations were calculated using a standard curve prepared with known concentrations of H_2O_2 .

Lipid peroxidation (thiobarbituric acid reactive substances (TBARS) content) was determined according to Rao and Sresty (2000). TBARS concentration was calculated from the absorbance at 532 nm, and measurements were corrected for nonspecific turbidity by subtracting the absorbance at 600 nm. The concentration of TBARS was calculated using an extinction coefficient of $155 \text{ mM}^{-1} \text{ cm}^{-1}$.

Identification of Isozyme and/or Enzyme Compositions

500 mg of fresh leaf tissue was homogenized in Tris-HCl (25 mM Tris, 1% Triton-X100, pH: 7.4) and centrifuged at 14,000 g for 30 min. Supernatants were collected, and total protein contents were measured by Bradford (1976) method. The native-PAGE was conducted on enzyme extracts to interpret the isozyme action (Laemmli 1970). The Gel Doc XR+ System was used to photograph stained gels and subsequently evaluated using Image Lab software v4.0.1 (Bio-Rad). For normalization, known enzyme standards are used in gels.

Identification of Isozyme and/or Enzyme Compositions

SOD (EC 1.15.1.1) isozyme/enzyme activities were measured (Beauchamp and Fridovich 1971; Laemmli 1970). CAT isozymes were detected according to Woodbury et al. (1971). Total CAT (EC 1.11.1.6) activity was estimated according to the method of Bergmeyer (1970). POX (EC 1.11.1.7) isozymes/enzyme activities were measured (Herzog and Fahimi 1973; Seever et al. 1971). The isozyme/enzyme activity of NADPH oxidase (NOX, EC 1.6.3.1) was calculated (Jiang and Zhang 2002; Sagi and Fluhr 2001). The isozyme/enzyme capacities of glutathione S-transferase (GST, EC:2.5.1.18) and glutathione peroxidase (GPX, EC:1.11.1.9) were determined (Hossain et al. 2006; Ricci et al. 1984).

Identification of Enzyme/Non-enzyme Compositions Related to AsA-GSH Cycle

For enzyme or isozyme activity, APX (EC 1.11.1.11) and GR (EC 1.6.4.2) were measured (Foyer and Halliwell 1976; Mittler and Zilinskas 1993; Nakano and Asada 1981). Dehydroascorbate reductase (DHAR; EC 1.8.5.1) and monodehydroascorbate reductase (MDHAR; EC 1.6.5.4) were assayed (Dalton et al. 1986; Miyake and Asada 1992). The

contents of ascorbate (AsA) and the oxidized form of ascorbate (DHA, dehydroascorbate) were performed (Dutilleul et al. 2003). The glutathione (GSH) and oxidized glutathione (GSSG) were determined (Paradiso et al. 2008). GSH redox state (GSH/GSH+GSSG) was calculated (Shi et al. 2013).

SOD, CAT, POX, GST, APX, GR and NOX isozyme bands were visualized and analyzed by the Gel Doc XR+ System and Image Lab software v4.0.1 (Bio-Rad). 0.5 units of SOD and 0.2 units of POX were used as standard amounts of enzymes.

Statistical Analysis

The data were expressed as mean values with standard errors. The Kolmogorov–Simirnov test and Levene's test were used to ensure the normality assumption and the homogeneity of variances, respectively. The experiments were repeated thrice independently and each data point was the mean of six replicates. All data obtained were subjected to a one-way analysis of variance (ANOVA). Statistical analysis of the values was performed by using SPSS 20.0. Tukey's post-test was used to compare the treatment groups. Comparisons with $p < 0.05$ were considered significantly different. In all figures, the error bars represent the standard errors of the means. The variation from low levels to high ones in the parameters obtained from the OJIP analysis compared to the control group was depicted on the color scale from blue to red.

Results

Water Content, Proline Content and Growth

As shown in Fig. 1A, the elevated CO_2 concentration (eCO_2) improved RWC by 24% in Col-0 under heat stress, while *srt2* mutants showed only minor improvement. In response to heat stress (H), a reduction in RWC was observed in wild-type and *srt2* mutant of *Arabidopsis* by 28% and 20%, respectively. When compared to the stress alone, eCO_2 -applied *Arabidopsis* plants had the high levels of RWC by 24% and 19% increase ($p < 0.05$). The addition of sirtinol (S) caused a reduced water content in wild-type plants. This trend of S application on RWC was detected under both eCO_2 and eCO_2 +H in Col-0. The stress exposure to Col-0 and *srt2* plants resulted in a significant reduction of Pro content during experimental period and the maximum decrement in Pro was in *srt2* mutants (by a 34% decline) (Fig. 1B). While eCO_2 under the control conditions did not occur a change in Pro of Col-0, the high content in Pro was observed under eCO_2 +H compared to the H alone in Col-0 plants. After S exposure under the non-stress or H stress, no

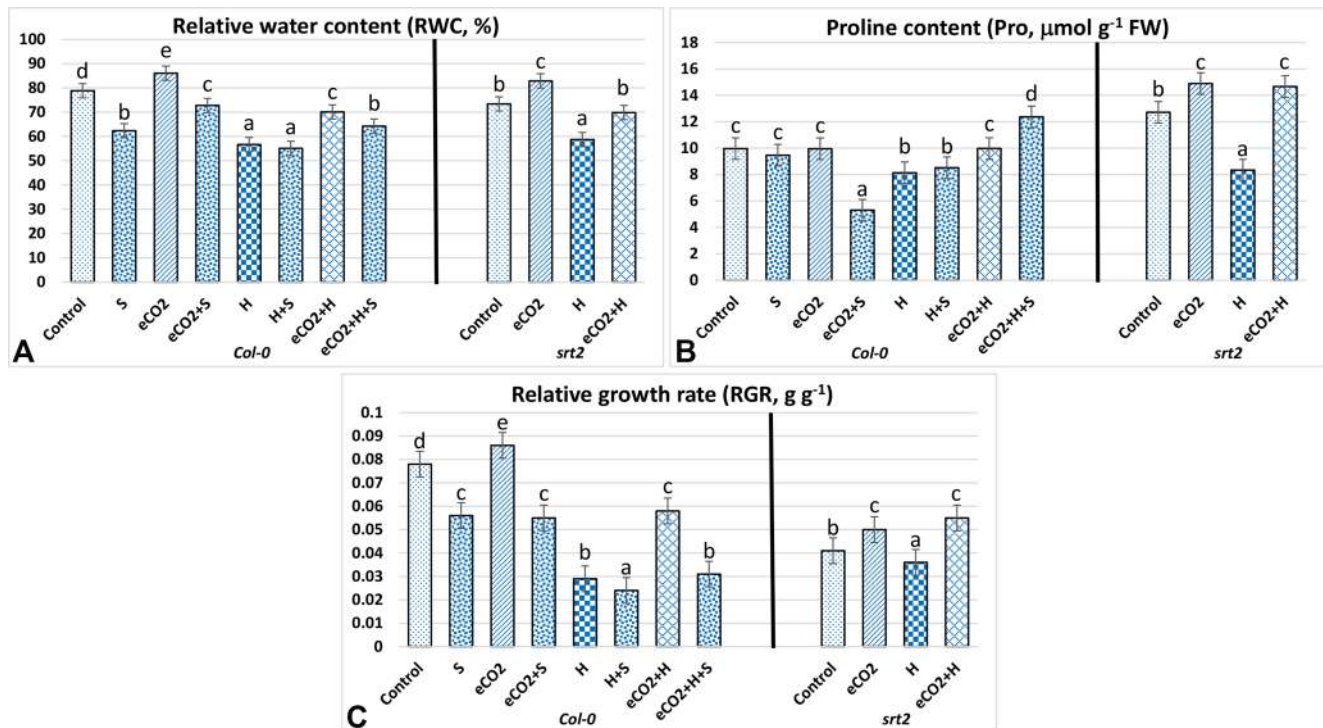


Fig. 1 The relative growth rate (RWC, A), proline content (Pro, B) and the relative growth rate (RGR, C) after the elevated CO₂ exposure (eCO₂: 800 ppm) under heat stress (H, 36 °C) in wild type cultivar Columbia-0 (Col-0) and SRT2-deficient mutant (*srt2*) of *Arabidopsis thaliana* for 24 h. C: Control, S: 1 μM Sirtinol, eCO₂: Elevated CO₂

change in Pro content was detected compared to the control group or stress alone in Col-0, respectively. On the other hand, in *srt2* mutant, eCO₂ application caused an induction of Pro content under both the control and stress conditions. As detected in Fig. 1C, in the presence of eCO₂, RGR increased in Col-0 and *srt2* plants. H-applied *Arabidopsis* plants exhibited low levels of RGR. eCO₂ had an alleviation effect on RGR against heat stress by a 2- and 1.5-fold increase ($p < 0.05$) in wild-type and *srt2* mutant plants, respectively. The inhibitor application for sirtuin resulted in the low RGR levels under the control or stress treatment in Col-0. As compared to eCO₂ and eCO₂+H, the reduced RGR was detected by S application.

Gas Exchange

Depending on eCO₂ treatment, the decline rate in g_s was higher in Col-0 (8-fold) than that of *srt2* plants (Fig. 2A). Similarly, a significant decline in g_s was observed under H stress in *Arabidopsis* plants. Both Col-0 and *srt2* exposed to H stress had the high levels of g_s by eCO₂ application. In Col-0, no change in g_s occurred under S, eCO₂+S or H+S compared to the control group, eCO₂ or H, respectively. As detected in Fig. 2B, the carbon assimilation rate (A) increased in the wild-type and mutant plants of *Arabidopsis*

concentration; 800 ppm, eCO₂+S: 800 ppm CO₂ concentration+1 μM sirtinol, H: Heat stress (36 °C), H+S: Heat stress+1 μM sirtinol, eCO₂+H: 800 ppm CO₂ concentration+Heat stress, eCO₂+H+S: 800 ppm CO₂ concentration+Heat stress+1 μM Sirtinol

treated with eCO₂ that was more obvious in Col-0. The presence of H stress caused a considerable decrease in A level as compared to the control group in Col-0 and *srt2*, but an enhancement in A of Col-0 was calculated under eCO₂ plus H. In this treatment, the change in A of *srt2* was similar to that of stress alone. S addition to ecotype Col-0 under the control or stress conditions significantly decreased the carbon assimilation. When compared to eCO₂+H, the alleviation effect of eCO₂ against stress on A was eliminated by S application. As shown in Fig. 2C, when compared to the control plants, the elevated levels of CO₂ in *Arabidopsis* significantly decreased C_i . H stress caused a decrement in C_i values of both *Arabidopsis* plants that detected the maximum reduction in *srt2* mutant by a 23% decline ($p < 0.05$). While no change in C_i of *srt2* plants observed under eCO₂+H as compared to the stress alone, eCO₂ together with H resulted in an induction in Col-0 during the experimental period. However, no reaction in C_i was followed by S application under the control, stress or eCO₂ conditions in Col-0 plants. After eCO₂ exposure, a decline in E levels was observed at similar levels in both wild type and mutant plants (by 3.6- and 3.1-fold declines ($p < 0.05$), respectively) (Fig. 2D). Similarly, in response to heat stress, E decreased and the maximum reduction was in *srt2* mutants by a 63% decline. Under eCO₂ or H conditions, S application did

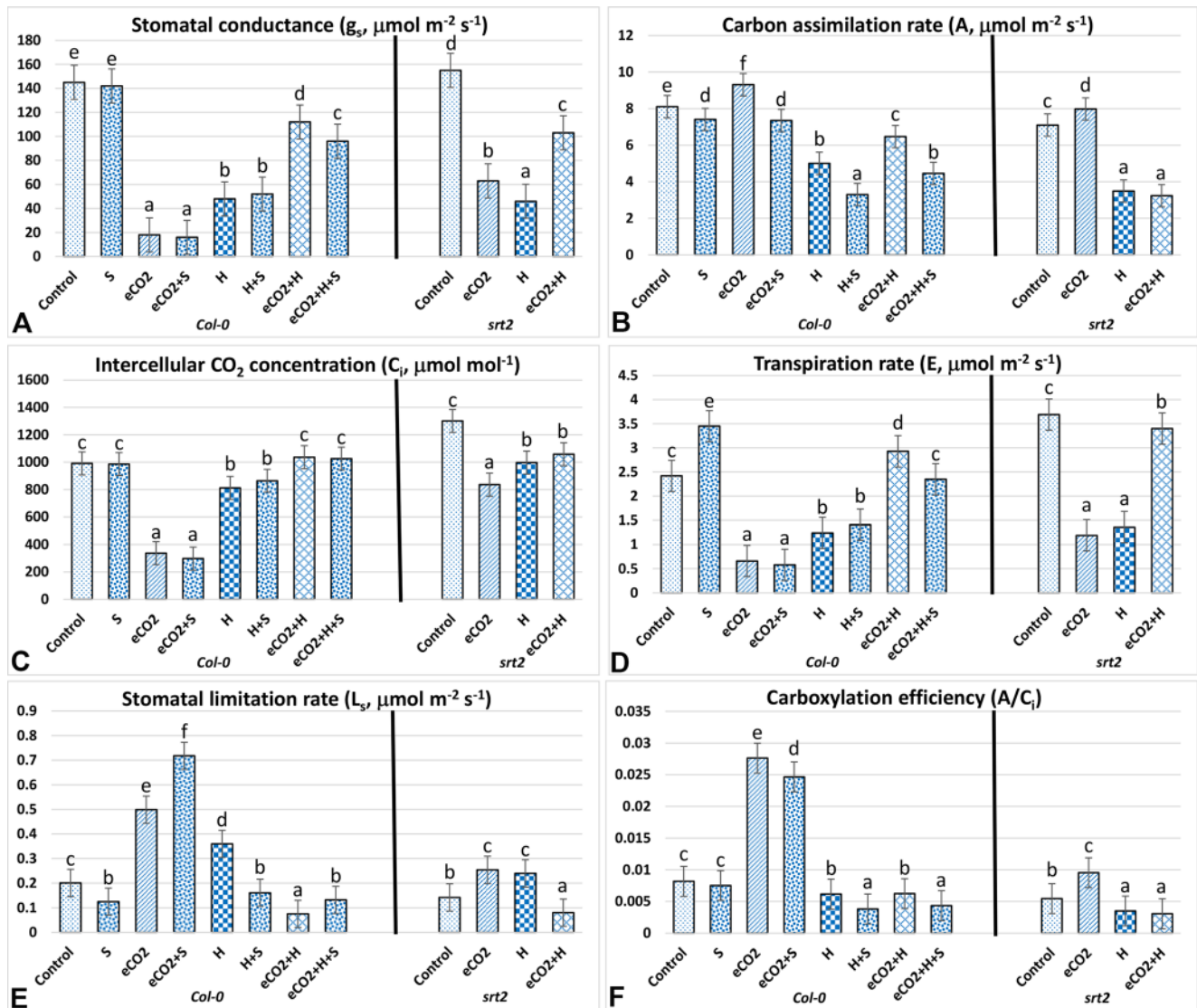


Fig. 2 Stomatal conductance (g_s , **A**), carbon assimilation rate (A, **B**), intercellular CO₂ concentrations (C_i , **C**), transpiration rate (E, **D**), stomatal limitation rate (L_s , **E**) and the carboxylation efficiency (A/ C_i , **F**) after the elevated CO₂ exposure (eCO₂: 800 ppm) under heat

stress (H, 36 °C) in wild type cultivar Columbia-0 (Col-0) and SRT2-deficient mutant (*srt2*) of *Arabidopsis thaliana* for 24 h. The definition of groups was given in Fig. 1

not alter E levels compared to the eCO₂ or stress, respectively. On the other hand, the high levels of E induced by eCO₂+H were reduced through S addition. The *Arabidopsis* plants treated with eCO₂ or stress treatment under the control conditions exhibited an increase in stomatal limitation (L_s) (Fig. 2E). However, compared with the control group, the limitation on stomata triggered by H treatment was removed by eCO₂ application. The limited rates on stomatal regulation increased in S-applied plants under eCO₂ and eCO₂+stress treatments. The carboxylation efficiency (A/ C_i) reached the high levels in both *Arabidopsis* plants exposed to eCO₂ application (Fig. 2F). The low rates in A/ C_i were observed in Col-0 and *srt2* mutant under stress. Interestingly, the combination of eCO₂ and H stress did not show

any change in A/ C_i compared to the stress alone. Under the control conditions, S caused no alteration in A/ C_i of Col-0 plants. S addition to eCO₂, H or eCO₂+H-applied ecotype Col-0 decreased A/ C_i .

Chlorophyll Fluorescence

As shown in Fig. 3A, there was no difference between eCO₂ treatment and the control group in F_v/F_m of both *Arabidopsis* plants. On the other hand, the decrease in F_v/F_o of *Arabidopsis* was triggered with eCO₂ application as compared to the control group (Fig. 3B). Heat stress inhibited F_v/F_m in Col-0 and *srt2* mutants by 14% and 12% declines ($p < 0.05$). Similarly, H-reduced F_v/F_o was calculated in Col-0 and *srt2*

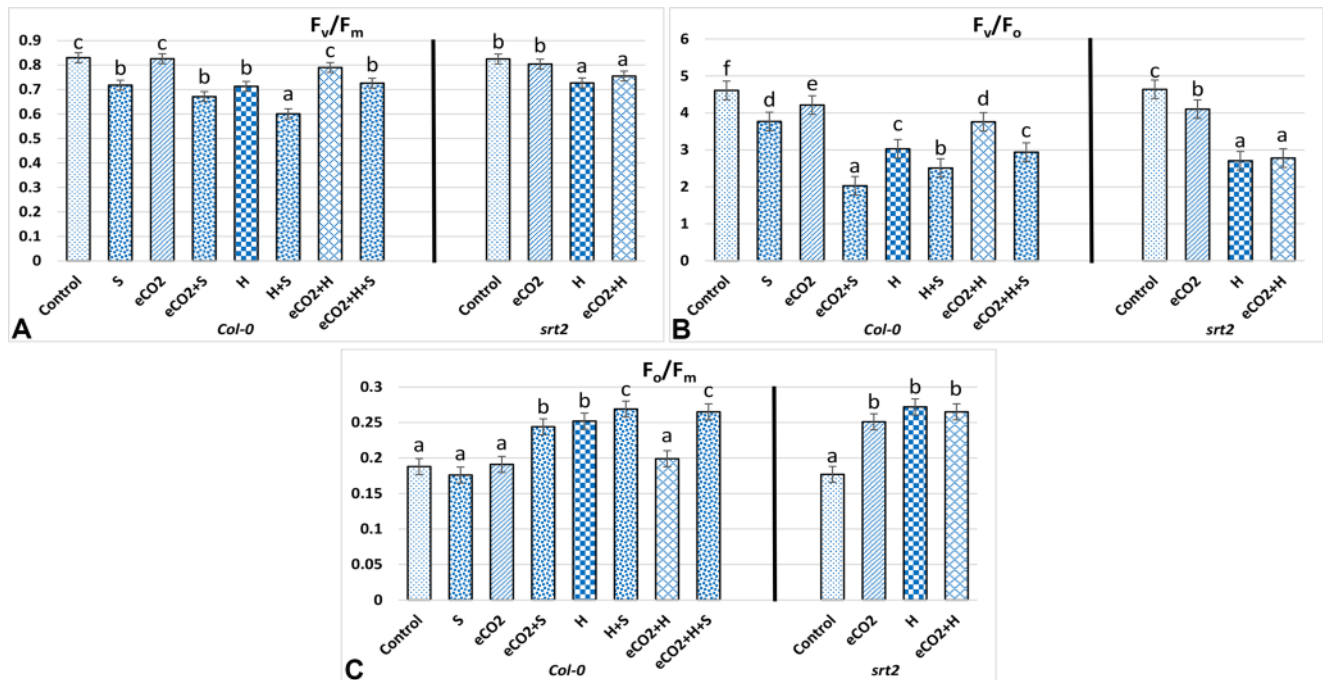


Fig. 3 The maximal quantum yield of PSII photochemistry (F_v/F_m , **A**), the potential photochemical efficiency (F_v/F_o , **B**) and the physiological state of the photosynthetic apparatus (F_o/F_m , **C**) after the elevated CO₂

mutants (Fig. 3B) and the maximum reduction (42%) was observed in H-applied *srt2* plants. A remarkable increment was measured in F_v/F_m and F_v/F_o of Col-0 plants under eCO₂ together with H stress compared to the stress alone, but it did not observe in *srt2*. The decrement in F_v/F_m and F_v/F_o was detected by S addition under control conditions, eCO₂, H stress or eCO₂+stress. As depicted in Fig. 3C, after eCO₂ exposure, only *srt2* exhibited the high ratios of F_o/F_m during the experimental period. There was a noticeable increase in F_o/F_m under stress and the value reached the maximum levels in *srt2* mutants (54% increase). The wild type *Arabidopsis* plants treated with eCO₂ plus H had lower F_o/F_m than the stress ones by 1.3-fold decline ($p < 0.05$). This trend did not observe in *srt2* mutants. On the other hand, the positive alteration in F_o/F_m did not maintain in Col-0 plants exposed to S treatment under eCO₂, H or eCO₂+H.

PSII Photochemistry

As detected in Fig. 4, compare to the control group, the elevated levels of CO₂ concentration did not significant alteration on ET_o/RC , TR_o/RC , DI_o/RC , $\Psi E_o/(1-\Psi E_o)$, $\gamma RC/(1-\gamma RC)$, V_J and photosynthetic performance (PI_{ABS} and PI_{total}) in Col-0 plants. However, the *SRT2*-deficient mutants had an increase in ABS/RC , dV/dt_o , V_I , V_J and decrease in ϕR_o and photosynthetic performance under eCO₂ conditions. Heat stress led to a considerable decrease in ET_o/RC , $\Psi E_o/(1-\Psi E_o)$, $\phi P_o/(1-\phi P_o)$, $\gamma RC/(1-\gamma RC)$, ΨE_o , ϕR_o , PI_{ABS}

exposure (eCO₂: 800 ppm) under heat stress (H, 36 °C) in wild type cultivar Columbia-0 (Col-0) and *SRT2*-deficient mutant (*srt2*) of *Arabidopsis thaliana* for 24 h. The definition of groups was given in Fig. 1

and PI_{total} under both *Arabidopsis* plants. On the other hand, the elevated levels of ABS/RC , TR_o/RC , DI_o/RC , dV/dt_o , V_I and V_J were detected in stress-treated plants as compared to the control group. In response to stress, these photosynthetic parameters showing specific and phenomenological energy fluxes, electron transfer and performance indexes were affected in a positive way by eCO₂ application. S addition to non-stress-, stress- or eCO₂-applied Col-0 plants reversed these impacts on photosynthesis.

H₂O₂ Accumulation and Lipid Peroxidation

No alteration in H₂O₂ content (Fig. 5A) and lipid peroxidation (TBARS content, Fig. 5B) was detected in Col-0 and *srt2* mutants treated with eCO₂. Stress caused an induction in H₂O₂ accumulation in plants, which the enhancement rate in H₂O₂ was higher in Col-0 (2.2-fold increase, ($p < 0.05$)) than in mutant plants. Similarly, compared to the control group, stress-applied wild-type and *srt2* mutants of *Arabidopsis* exhibited the high levels (25% and 80% increase ($p < 0.05$), respectively) in TBARS content. eCO₂ application together with H stress decreased H₂O₂ content in both *Arabidopsis* plants. This trend was also detected in TBARS of Col-0 and *srt2* mutants by 1.5- and 1.1-fold decline, respectively (Fig. 5B). This alleviation on H₂O₂ and TBARS contents was removed by S application in Col-0 plants and even S-exposed wild type plants had an induction in these contents.

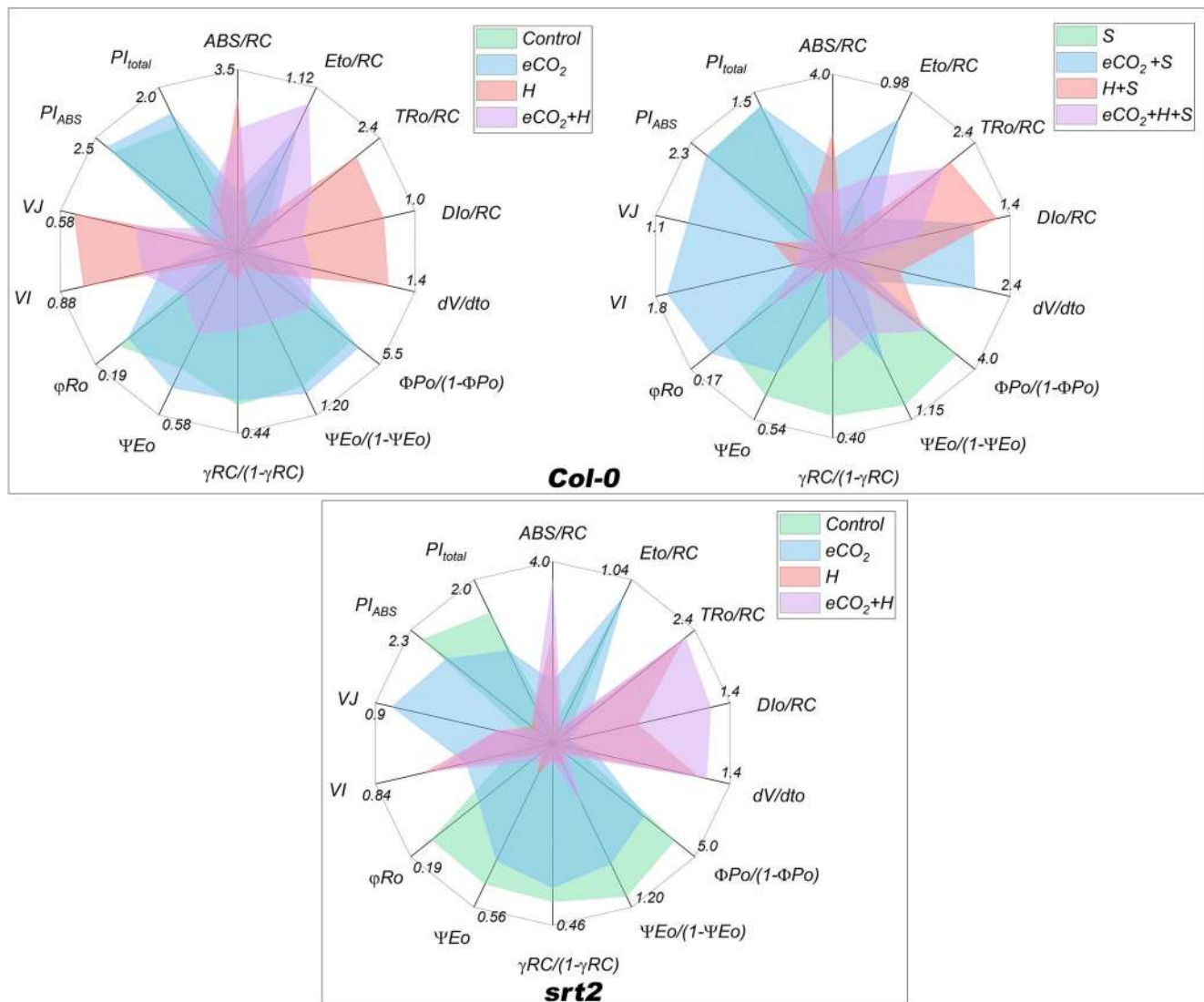


Fig. 4 The average absorption per active reaction centers (ABS/RC), electron transport flux per active reaction centers (ET_o/RC), flux or exciton trapped per active reaction center (TR_o/RC), Q_A-reducing RCs per photosystem II antenna chlorophyll ($\Phi P_o/(1-\Phi P_o)$), the efficiency of the trapped exciton to transfer an electron to the photosynthetic electron transfer chain ($\Psi E_o/(1-\Psi E_o)$), Q_A⁻ reducing reaction centers per photosystem II antenna chlorophyll ($\gamma RC/(1-\gamma RC)$), ratio of total dissipation to the amount of active reaction center (DI_o/RC), performance

index based on the absorption of light energy (PI_{ABS}) and performance index for energy savings from excitons to the reduction of photosystem I and acceptors (PI_{total}) after the elevated CO₂ exposure (eCO₂: 800 ppm) under heat stress (H, 36 °C) in wild type cultivar Columbia-0 (Col-0) and SRT2-deficient mutant (*srt2*) of *Arabidopsis thaliana* for 24 h. The parameters derived from the portable fluorometer and OJIP transient and their definitions for use in the current study were given in Supplementary Table S1. The definition of groups was given in Fig. 1

Antioxidant Enzyme Activity

Figure 6A revealed that four SOD isozymes (one band for Mn-SOD and Cu/Zn-SOD and, two bands for Fe-SODs in Col-0 and four bands for Mn-SODs and one band for Cu/Zn-SOD in *srt2* mutant) were detected in *Arabidopsis* leaves. After eCO₂ exposure, there was an induction in total SOD activity in wild type and mutant (Fig. 6A). These changes were proved by the gel staining analysis for Fe-SODs and Cu/Zn-SOD in Col-0 and Mn-SOD2-3-4 and Cu/Zn-SOD in *srt2* mutant plants (Fig. 6B). Similarly, there

was a significant increment in the total SOD activity of stress-treated *Arabidopsis* plants. This trend was also maintained by eCO₂ application together with stress compared to stress alone. This response of eCO₂ under stress on SOD activity was parallel to the intensities of SOD isozymes. While S addition to eCO₂ plus stress-applied Col-0 plants decreased SOD activity, no change was observed in plants with S, eCO₂+S and HS compared to the control, eCO₂ and stress alone, respectively. As shown in Fig. 6C, two CAT bands in Col-0 and only one CAT band in *srt2* was appeared during experimental period. eCO₂ application did not cause

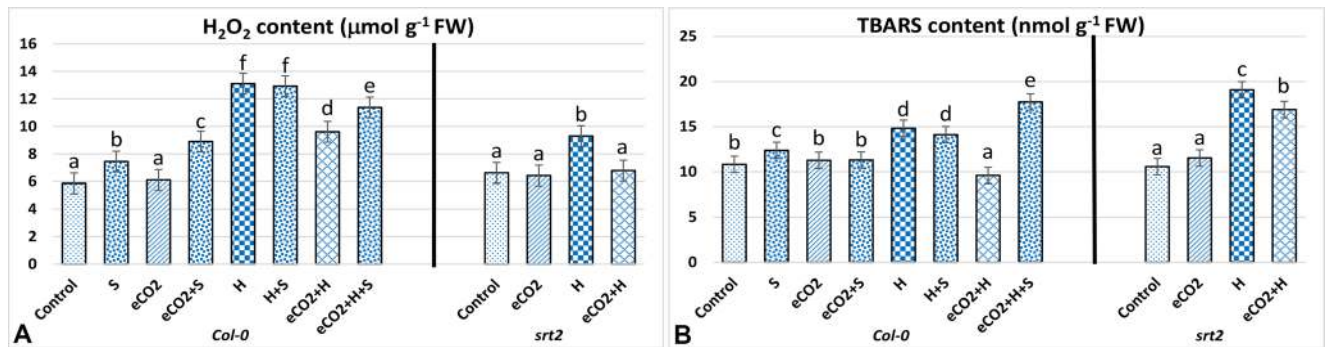


Fig. 5 Hydrogen peroxide content (H₂O₂, **A**) and lipid peroxidation (TBARS, **B**) after the elevated CO₂ exposure (eCO₂: 800 ppm) under heat stress (H, 36 °C) in wild type cultivar Columbia-0 (Col-0) and

SRT2-deficient mutant (*srt2*) of *Arabidopsis thaliana* for 24 h. The definition of groups was given in Fig. 1

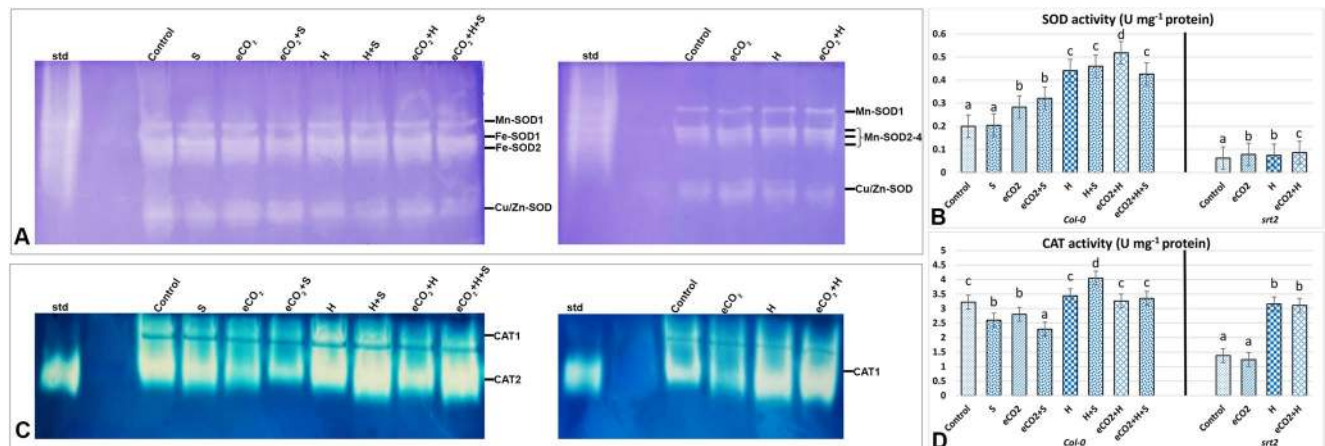


Fig. 6 Relative band intensity of different types of superoxide dismutase isoenzymes (SOD, **A**) and total SOD activity (**B**), relative band intensity of different types of catalase isoenzymes (CAT, **C**) and total CAT activity (**D**) after the elevated CO₂ exposure (eCO₂: 800 ppm)

under heat stress (H, 36 °C) in wild type cultivar Columbia-0 (Col-0) and SRT2-deficient mutant (*srt2*) of *Arabidopsis thaliana* for 24 h. The definition of groups was given in Fig. 1

an increase in the total CAT activity (Fig. 6D). In response to H stress, *srt2* leaves exhibited an increment in this activity by 2.3-fold increase ($p < 0.05$), but not in Col-0 plants. CAT activity of the stressed-plants grown under eCO₂ conditions remained the same level to the stress alone group. This trend was provided through the intensity of CAT isoform. While S application caused an induction in CAT in response to H stress, this activity decreased after S, eCO₂+S or eCO₂+H+S in Col-0 plants.

As shown in Fig. 7A, in gel analysis for POX isoforms, three isozymes (POX1-3) were visualized in both *Arabidopsis* cultivars. The wild-type *Arabidopsis* plants exposed to eCO₂ had lower POX activity, as indicated by reduced intensity of POX1 band. However, this activity in *srt2* mutant was greater under eCO₂ conditions as compared to the control group (Fig. 7B). This induction was associated with the increased intensity of POX2 isozyme. The lower POX activities were recorded in both *Arabidopsis* plants treated with H stress. The combined exposure of eCO₂ and H stress in Col-0 and *srt2* mutants caused the high POX

activity, when compared to the stress alone. In the wild type plants, the reduced POX activity was found when S applications together with eCO₂ or eCO₂+H stress. On the other hand, the presence of sirtinol increased POX activity after stress exposure by 25% increase ($p < 0.05$). During the experimental period, four bands in Col-0 and three bands in mutant plant regarding the APX isoforms were defined (Fig. 7C). There was no noticeable difference in APX activity in eCO₂-exposed *srt2* mutant. Both APX enzyme activity and the isozyme staining pattern (APX1-4) exhibited a decrement in Col-0 plants with eCO₂ application. H stress suppressed the APX activity in both *Arabidopsis* plants (Fig. 7D), but the presence of eCO₂ was removed the reduction of this activity. After eCO₂ or H stress exposure, S addition to the wild-type plants maintained the high APX activity.

Gel analysis reveals that six and five GR isozymes are observed in treatment groups of Col-0 and *srt2* mutant, respectively (Fig. 8A). Result shown in Fig. 8B suggests that there is a trend towards increasing activity of GR

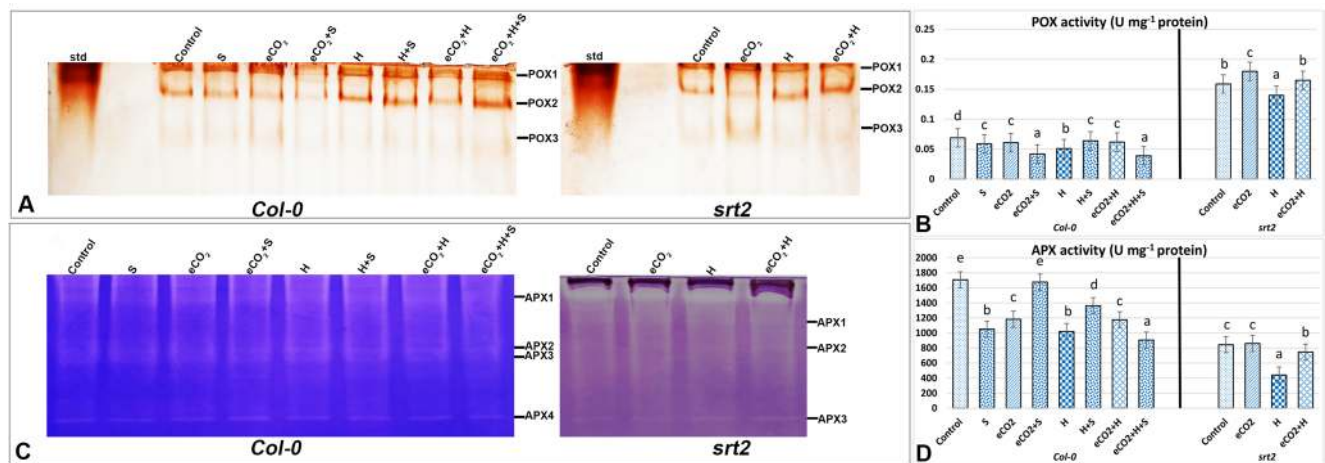


Fig. 7 Relative band intensity of different types of peroxidase isoenzymes (POX, A) and total POX activity (B), relative band intensity of different types of ascorbate peroxidase isoenzymes (APX, C) and total APX activity (D) after the elevated CO₂ exposure (eCO₂: 800 ppm)

under heat stress (H, 36 °C) in wild type cultivar Columbia-0 (Col-0) and SRT2-deficient mutant (*srt2*) of *Arabidopsis thaliana* for 24 h. The definition of groups was given in Fig. 1

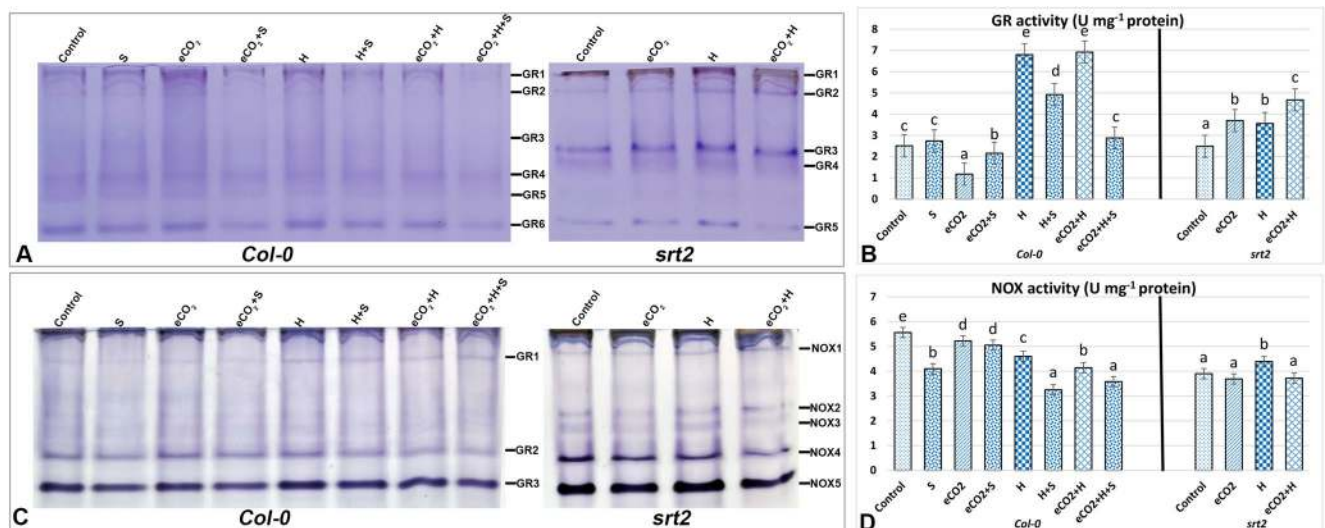


Fig. 8 Relative band intensity of different types of glutathione reductase isoenzymes (GR, A) and total GR activity (B), relative band intensity of different types of NADPH oxidase isoenzymes (NOX, C) and total NOX activity (D) after the elevated CO₂ exposure (eCO₂: 800

ppm) under heat stress (H, 36 °C) in wild type cultivar Columbia-0 (Col-0) and SRT2-deficient mutant (*srt2*) of *Arabidopsis thaliana* for 24 h. The definition of groups was given in Fig. 1

enzyme in *srt2* mutant subjected to eCO₂ application, but not in Col-0. Stress activated GR activity, which the wild type cultivar treated with H stress had a higher GR activity of than the control group by 2.7-fold increase ($p < 0.05$) (Fig. 8B). GR1-6 and GR1-5 isozymes were responsible for this induction of Col-0 and mutant plants, respectively (Fig. 8A). Only *srt2* mutant protected the induced GR activity under the combination of eCO₂ and H stress, as compared to the stress alone. S addition to the control group, H-applied or eCO₂+H-treated plants did not provide an induction in GR activity of Col-0 plants. Gel analysis for observing NOX isoforms presents three and five NOX bands in the *Arabidopsis* leaves, respectively (Fig. 8C).

There was no increase in NOX activity of both *Arabidopsis* plants treated with eCO₂ application (Fig. 8D), as supporting the NOX isoform activities. In contrast to this result, it was found that after H exposure the high activity of NOX was observed in *srt2* mutant. Similar to the total enzyme activity, the intensities of NOX3-4 isoforms were stronger under stress group than that of the control one (Fig. 8C). A significant drop in NOX was observed in response to the combined treatment of eCO₂ and H stress in *Arabidopsis*. S-reduced NOX activity was observed under S, H+S and eCO₂+H+S groups in the wild-type *Arabidopsis* plants.

In this study, two and four GST isoforms were detected by native page analysis (Fig. 9A). eCO₂ or H stress-exposed

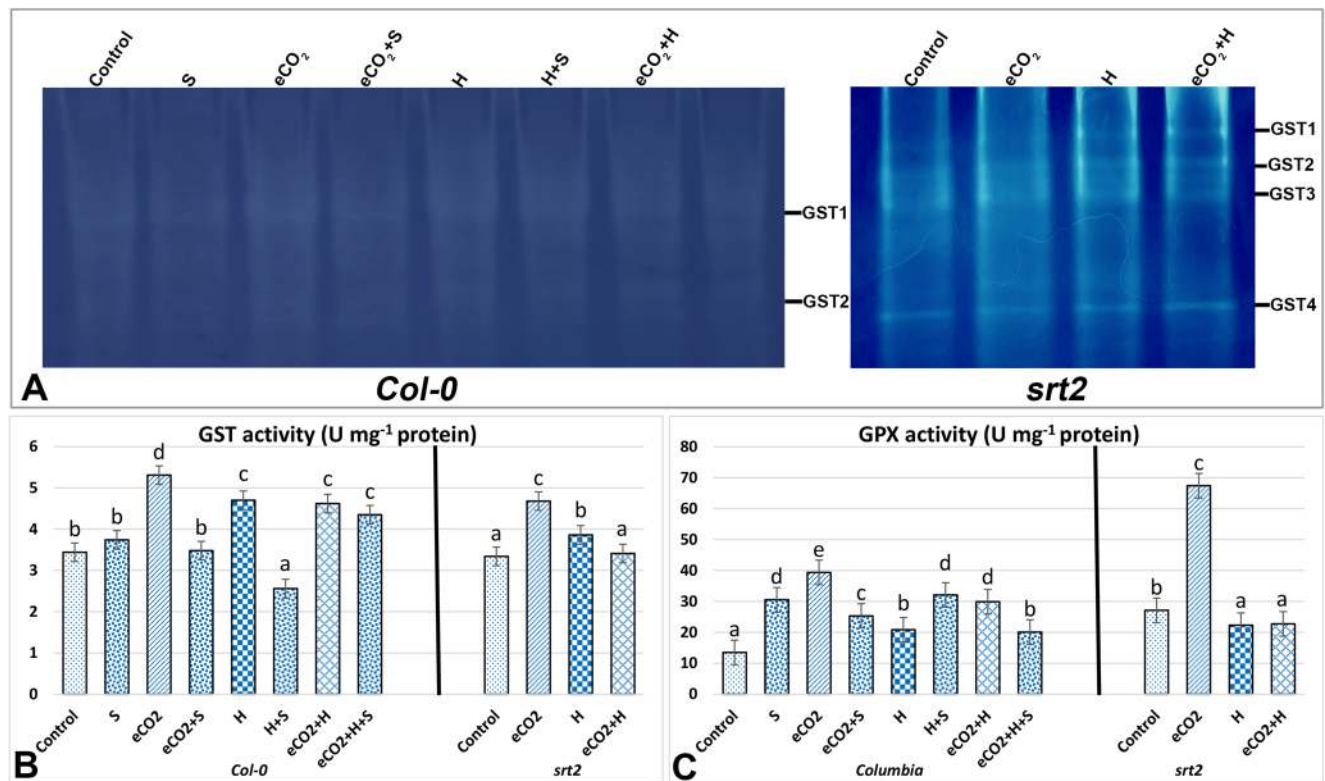


Fig. 9 Relative band intensity of different types of glutathione S-transferase isoenzymes (GST, A) and total GST activity (B) and total glutathione peroxidase activity (GPX, C) after the elevated CO₂ exposure

(eCO₂: 800 ppm) under heat stress (H, 36 °C) in wild type cultivar Columbia-0 (Col-0) and SRT2-deficient mutant (*srt2*) of *Arabidopsis thaliana* for 24 h (h)

plants exhibited the high GST activity (Fig. 9B). The intensities of all GST isozymes proved this increase in *srt2* mutants. The greatest GST activity (5.31 U mg⁻¹ protein) was recorded in eCO₂-treated Col-0 plants (by 1.5-fold increase ($p < 0.05$)). S addition to Col-0 plants removed the eCO₂ or H-induced changes on GST activity. In contrast to this result, eCO₂ application under H stress conditions had lower or unchanged GST activity than the stress alone in *Arabidopsis* plants. eCO₂ application caused an increase on GPX activity of Col-0 and *srt2* mutants, which was a maximum induction in mutant plants by 2.5-fold ($p < 0.05$). This trend in GPX was only detected in H-applied Col-0 plants. The elevated CO₂ levels against H stress protected the induced GPX activity. S addition decreased this protection in eCO₂+H-treated plants on GPX activity.

After eCO₂ exposure, MDHAR and DHAR activities increased in Col-0 (Fig. 10A) and *srt2* mutants (Fig. 10B), respectively. Both MDHAR and DHAR were suppressed by the H stress, which was more obvious in MDHAR of Col-0 and DHAR of *srt2* mutants. Interestingly, the H-mediated suppressed MDHAR and DHAR activities was increased by eCO₂ application in response to stress. This change in eCO₂+H-applied Col-0 plants was removed by S application. As shown in Fig. 10C and D, only eCO₂-treated Col-0 plants exhibited the high contents of tAsA and DHA, but not

in *srt2* mutant. Depending on these contents, the induced tAsA/DHA levels were calculated in these treatment group (Fig. 10E). While the lower contents on tAsA and DHA were detected under H stress, both *Arabidopsis* plants had a higher accumulation of DHA content. The combination of eCO₂ and H stress stimulated the tAsA contents in Col-0 and mutant and the induction rate of tAsA/DHA was by 4.2- and 2-fold rise ($p < 0.05$). S addition to Col-0 plants eliminated the positive effects of eCO₂ and eCO₂+H applications on this ratio (Fig. 10E). A significant enhancement was measured in GSH and GSSG contents of eCO₂-treated *Arabidopsis*, except for GSSG content in *srt2* mutant as compared to the control group (Fig. 10F and G). While GSH content decreased in the presence of stress, GSSG content triggered in both *Arabidopsis* plants. When based on these contents, GSH/GSSG and GSH redox state (Fig. 10H) was dropped by stress treatment. Interestingly, during the experimental period, there was no effect on GSH content in the wild type plants with eCO₂+H application as compared to the stress alone. So, eCO₂+H did not occur a significant effect on GSH/GSSG and GSH redox state in Col-0. However, there was an opposite stimulation in *srt2* mutant, which reached the maximum levels in GSH/GSSG and GSH redox state by 8- and 1-fold increase ($p < 0.05$), respectively. S addition to eCO₂- or H-applied plants decreased GSH content.

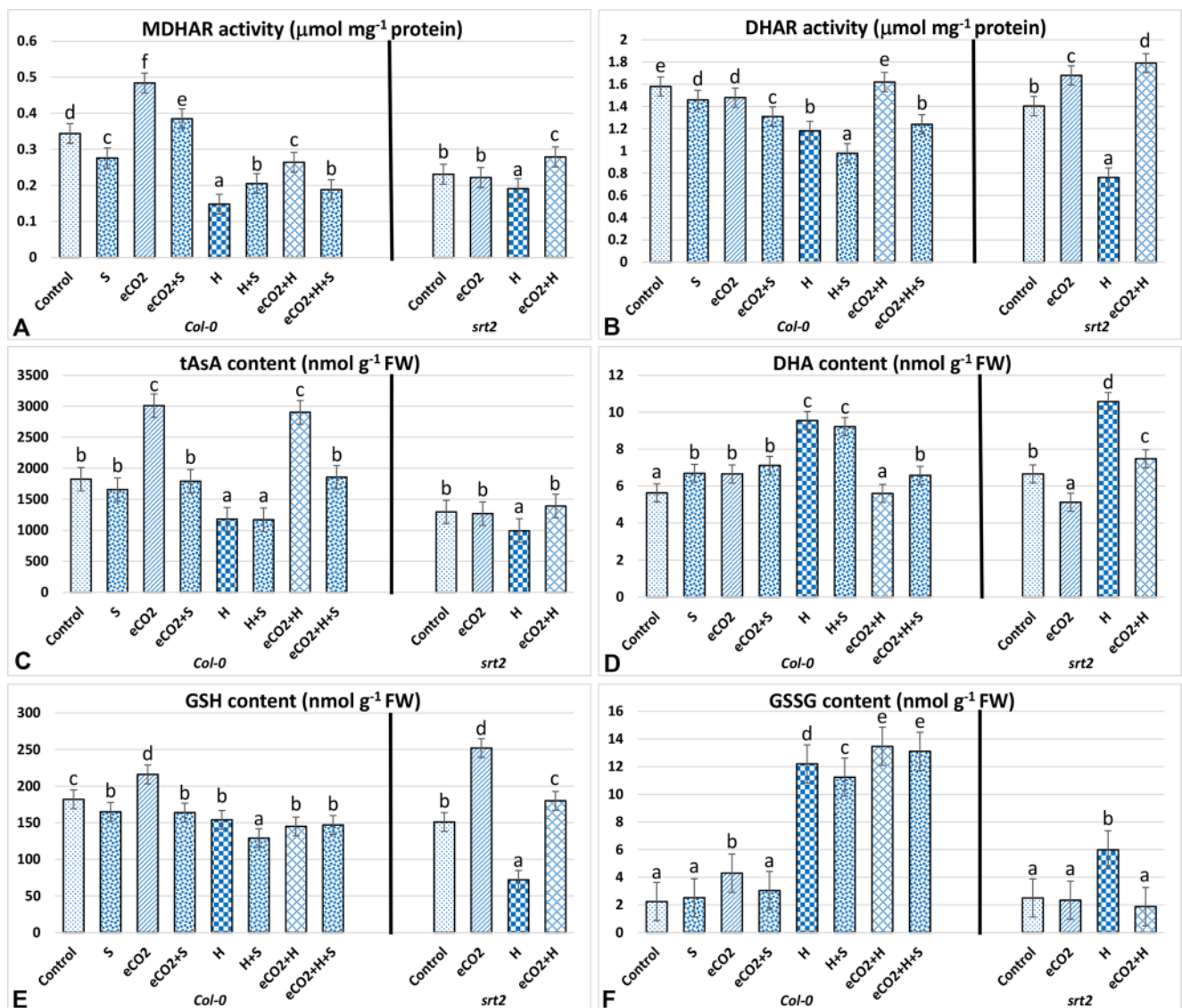


Fig. 10 Monodehydroascorbate reductase activity (MDHAR, **A**), dehydroascorbate reductase activity (DHAR, **B**), total ascorbate content (tAsA, **C**), dehydroascorbate content (DHA, **D**), glutathione content (GSH, **E**), oxidized glutathione content (GSSG, **G**), and GSH

redox state (**H**) after the elevated CO₂ exposure (eCO₂: 800 ppm) under heat stress (H, 36 °C) in wild type cultivar Columbia-0 (Col-0) and SRT2-deficient mutant (*srt2*) of *Arabidopsis thaliana* for 24 h. The definition of groups was given in Fig. 1

Considering eCO₂+S, H+S and eCO₂+H+S treatments, GSH/GSSG and GSH redox state were similar to the eCO₂, H or eCO₂+H.

Discussion

Growth, Water Management and Gas Exchange

As a basic consequence of stress, it is reported decreases in gas exchange components, such as stomatal conductance and transpiration rate, and biomass in plants (Ulfat et al. 2024). Consistent with this view, in our study, the lower *g_s*

and E levels of *Arabidopsis* plants exposed to H stress were stress responses and led to declines in photosynthetic efficiency. The stress-dependent low levels of photosynthetic products are related to reduction in RGR, as in the present study. On the other hand, Hollender and Liu (2008), König et al. (2014) and Cucurachi et al. (2012) reported that sirtuins function in mitochondrial energy metabolism, regulation of photosynthetic activity and auxin synthesis and, its transport. Our results proved that sirtinol (S) supplementation, a sirtuin inhibitor, to Col-0 plants caused a reduction in RGR, which could relate to the auxin impairment. S application further reduced RGR when applied with both stress and eCO₂. This finding was compatible with the data obtained

by Wang et al. (2024), who reported that SRT1-2 modulates growth of cultivated strawberry as well as resistance against heat stress. Besides, this growth decrease was related to the negative effect of S application on chlorophyll fluorescence. The elevated CO₂ levels have important impacts on carbon availability, photosynthesis, antioxidant systems and water-use efficiency by reducing stomatal resistance in plants (Shokat et al. 2021). Interestingly, Cai et al. (2020) reported decreases g_s in the presence of eCO₂, consistent with our findings, that this response is related to photosynthetic acclimation. Yang et al. (2023) also found that g_s was initially lower in tomato plants grown under eCO₂ compared to the control group, consistent with the common phenomenon that eCO₂ triggers stomatal closure thus lowering g_s . In the present study, the lowered g_s and E levels showed conserved water status in *Arabidopsis* plants grown under eCO₂ conditions resulting in a better RWC levels. The lowered E levels at eCO₂ by a reduction in g_s of both wild type and mutant plants of *Arabidopsis* could be a connection with the homeostatic regulation. There is a report showing that eCO₂-induced stomatal closure is to maintain water balance via aquaporin PIP2.1 in coffee plants (Avila et al. 2020). As mentioned by Wang et al. (2020), eCO₂-reduced g_s levels caused an enhancement resistance to entry of CO₂ into the leaf, thus partially offsetting the maximum stimulation of carboxylation rate. This case proved the results of C_i and A that under eCO₂ conditions against H stress, the higher C_i levels depending on stomatal opening (as showing increased g_s) induced the assimilation rates (A) and the growth rate (RGR) subsequently increased. High g_s values could permit *Arabidopsis* leaves cooling through evapotranspiration; which is associated with eCO₂ tolerance against heat stress. In spite of induced g_s levels, the high endogenous water status (increased RWC) in both *Arabidopsis* cultivars-applied-eCO₂ plus stress could explain with preserving water channel activity and Pro accumulation. In protecting the osmotic homeostasis of the stressed-plants, proline, as an osmoregulatory molecules, is an important player (Abuelsoud et al. 2023). Our findings indicated a positive correlation between RWC and Pro content, elucidate the significance that higher Pro content under eCO₂ helped the *Arabidopsis* plants to increasing tolerance against heat stress by stabilizing the antioxidant capacity. Gong et al. (2023) revealed that eCO₂ could enhance Pro accumulation by inhibiting proline dehydrogenase. In SRT-deficient *Arabidopsis* mutants, high proline accumulation also contributed to water regulation under stress or control conditions. Liu et al. (2017) reported that overexpression of SRT2 provided salinity tolerance by increasing Pro content in *Arabidopsis* plants. Similar to RWC content, eCO₂ could remove the restriction on growth (RGR) mediated by heat stress in *Arabidopsis* cultivars. As discussed by Wei et al.

(2024) in *Ormosia hosiei*, it might be the reason that eCO₂ concentration could promote carbon and nitrogen accumulation in plants exposed to stress. Abuelsoud et al. (2023) explained that eCO₂ promotes the sink strength and allocation of carbon and sugar content, which provide the necessary energy for division and expansion of cells and growth. Some studies reported the growth induction mediated by the elevated CO₂ levels via increased photosynthetic activity (Byeon et al. 2021; Zheng et al. 2020), while the other studies explained the higher growth rate through water status (Liu et al. 2019; Wei et al. 2021) under the control or stress conditions. Our study determined that eCO₂ enhances water relations of both *Arabidopsis* plants under heat stress because eCO₂ permits a greater osmotic adjustment capacity (increased Pro content) and a greater carbon supply from increased photosynthesis in spite of inductions in g_s and E. In response to eCO₂ plus stress in the wild type of *Arabidopsis* (Col-0), the result of greater carbon assimilation rate (A) was parallel to increased growth levels (RGR), as proved by Han et al. (2023). This can be explained by the increases in g_s and transpiration rate (improve the water supply to leaves of Col-0 cultivars via stomatal opening), thus allowing higher RWC levels.

PSII Photochemistry

To better interpret the changes on photosynthesis, we analyzed photosynthetic rate and PSII photochemistry. The ratio of variable fluorescence to maximum fluorescence (F_v/F_m) is a basic marker showing how effectively excitation energy is captured by opened reaction centers of PSII in the stressed plants (Jat et al. 2024). In the present study, H stress-applied Col-0 and *srt2* mutant plants exhibited a damage in the donor side of PSII and uncontrolled heat dissipation in PSII leading over-excitation of reaction centers, as evidenced by reduced F_m and F_v levels and high F_o values. In wild type cultivar of *Arabidopsis* (Col-0), the elevated CO₂ levels increase protected PSII from higher excess in excitation energy depending on heat stress, as showed through the higher F_v/F_m and F_v/F_o . The reduction in F_o/F_m of wild type plants of *Arabidopsis* associated with eCO₂-mediated decline in non-photochemical quenching under H stress. Interestingly, in spite of increasing in g_s and E of *srt2* mutant plants treated with eCO₂ and H stress, the remained levels of C_i and carboxylation efficiency (A/C_i) and decreased stomatal limitation rate could be explain unchanged status on F_v/F_m , F_v/F_o and F_o/F_m as compared to the stress alone. Besides, no observation increased in F_m (photochemical quenching) of eCO₂+H stress-applied *srt2* mutants, which means no improvement in the pool size of Q_A and inefficient electron transport of PSII donor side. In this situation, in the absence of SRT2 gene, *srt2* mutant, the

elevated treatment of CO₂ did not contribute to the alleviation in excessive excitation energy of PSII under H stress. These results on chlorophyll fluorescence and gas exchange in *srt2* mutants were proved by exogenous application of sirtinol to Col-0 plants. The different results in terms of chlorophyll fluorescence between Col-0 and *srt2* mutant indicated that the elevated CO₂ levels eliminated the negative effects of heat stress on PSII fluorescence in wild type of *Arabidopsis* or the SRT2 gene may interact with the rubisco-encoding genes or their expression levels under eCO₂+stress conditions. Besides, SRT2 gene identified in *Vitis vinifera* is reported in regulation of gene transcript levels and to be indirectly related to photosynthesis (Cucurachi et al. 2012). Previous reports showed a connection between decline in photosynthesis and lower rubisco efficiency (by reduced active states of Rubisco activase and transcript levels for the small subunit of rubisco (rbcS) under eCO₂ and stress conditions (Doron et al. 2020; Wei et al. 2022). The stronger correlations between chlorophyll fluorescence and PSII photochemistry indicated in Col-0 and *srt2* mutants of *Arabidopsis*. In H stress-treated *Arabidopsis* plants, the findings on chlorophyll a fluorescence exhibited a marked decrease in photosynthetic machinery and active PSII reaction centers, which is in line with that of Yamori et al. (2014). The higher values of ET₀/RC, TR₀/RC, DI₀/RC, V_I and V_J supported the increased number of inactive reaction centers, impaired oxygen-evolving complex and the declined status of re-oxidation to Q_B of reduced Q_A⁻ in the presence of stress conditions. As a parallel to the increased carbon assimilation levels (A) and carboxylation rate (A/C_i), eCO₂ condition independent of H stress could stimulate the demand of electron utilization in electron transport system of photosynthesis, which leads to reduced leakage of electrons from transport system. Also, eCO₂ together with H stress could alleviate imbalance on effective electron flow of PSII, chlorophyll content, absorption rate and electron flow per reaction centers, less damaged water splitting complex in PSII and trapping rate of absorbed photons, as proved by ABS/RC, ET₀/RC, DI₀/RC, TR₀/RC, performance indexes (PI_{ABS} and PI_{total}) when compared to the stress alone. In our study, the findings on PSII photochemistry indicated that the photoprotection in eCO₂-applied Col-0 plants could be linked to the protection of reaction centers. The findings reported by Kalaji et al. (2016) and Haque et al. (2022) on photosynthetic potential of PSII was in consonance with the present results. Under H stress, eCO₂ triggered the better conservation on operative photosynthetic system in Col-0 than that of *srt2* mutants. This situation can be interpreted as the *srt2* gene interacting with proteins related to photosystems (PSI or PSII).

ROS Accumulation and Antioxidant Capacity

Stress-exposed plants exhibit a decline in photosynthesis and an induction in light excitation energy. In this case, overexcitation of the photosynthetic pigments in the antenna triggers. The damages of photosynthetic apparatus led to excessive excitation energy in PSII, leading to excessive production of ROS (Muñoz and Munné-Bosch 2018). To diminish ROS-based impairments, the plants had antioxidant systems including SOD, CAT, APX, GR, ascorbate and glutathione (Khalid et al. 2023). Our results confirmed that together with reduction in RGR, RWC and photosynthetic efficiency, the induced activities (SOD and GR) and reduced levels (POX, APX, AsA/DHA and GSH redox state) point to increased H₂O₂ accumulation, which showed oxidative damage in both Col-0 and *srt2* mutant of *Arabidopsis*. Heat stress-treated *Arabidopsis* plants experienced oxidative stress as proved by the high levels of lipid peroxidation (TBARS content). Tang et al. (2022) indicated that salinity-induced damage rates in nucleus of *Arabidopsis* were higher in *srt2-1* and *srt2-2* than that of wild type plants. Previous data indicated the increased activity of antioxidant enzymes under combined treatment of eCO₂ and heat stress (Shokat et al. 2021). Elevated CO₂ suppresses photorespiration (Zinta et al. 2014) and results in less ROS (mostly H₂O₂) in the arsenic-stressed barley plants (Selim et al. 2021). Zinta et al. (2018) found that eCO₂-mediated mitigating effect on damages induced by stress might be related to increased levels of sugar, amino acids and antioxidant capacity. Our data on SOD activity, which is the first enzyme to eliminate superoxide anion radicals, showed that eCO₂+H-treated *Arabidopsis* plants (both Col-0 and *srt2*) had a dismutation of O₂⁻ to H₂O₂ by increased SOD activity. Similarly, our results clearly showed a remarkable decline regarding H₂O₂ accumulation, which contributed to POX and AsA regeneration (increased APX, MDHAR, DHAR, AsA/DHA) in Col-0 and POX and both AsA and GSH redox status (induced APX, GR, MDHAR, DHAR, AsA/DHA and GSH/GSSG) in *srt2* mutant plants. While, in this study, POX activity was dominant enzyme for scavenging H₂O₂ content in eCO₂ plus H stress in both *Arabidopsis* plants, a contrary trend is reported by Wang et al. (2023), who identified the low activity of POX under eCO₂ and lead stress in *Oryza sativa*. As well as induced antioxidant activity mediated by SRT2 gene, Qiong et al. (2022) indicated that SRT-like histone deacetylase can regulate the trafficking of H₂O₂-containing vesicles in salt-stressed *Arabidopsis* plants, which is resulted in reduced excessive H₂O₂ accumulation. As connection with this data, *Atsrt2-1* mutants exhibited a reduction in H₂O₂ accumulation by suppressing of VAMP714 (vesicle-associated membrane protein 714). and thereby producing salt stress tolerance (Tang et

al. 2022). Another report indicated that SRT1 overexpression enhanced tolerance to oxidative stress in *Oryza sativa* (Huang et al. 2007). Col-0 plants having impaired SRT gene by sirtinol application (S group) showed that sirtinol caused both the continuation of damage against H stress and further increased the level of lipid peroxidation (TBARS content) in eCO₂-mediated protection against stress damage. Besides, AsA accumulation in *Arabidopsis* plants with eCO₂ and H stress closely associated with carbohydrate metabolism and could alleviate PSII photochemistry, as previously presented by Zinta et al. (2014). On the other hand, as suggested by Bruscalupi et al. (2023), the plant-based sirtuins, like in animals, have been defined roles in metabolism regulation and stress defense. SRT1 gene is overexpressed in rice plants and plays a role in both genome stability and cellular redox balance provides tolerance when applied oxidative stress (Huang et al. 2007).

Conclusion

Based on the aforementioned findings, heat-stressed *Arabidopsis thaliana* plants demonstrated significant impairments in vegetative growth, relative water content, proline accumulation, and key chlorophyll fluorescence parameters (F_v/F_m , F_v/F_o , and F_o/F_m), indicative of photo inhibitory damage. Furthermore, gas exchange parameters—including net photosynthetic rate, intercellular CO₂ concentration, transpiration rate, and carboxylation efficiency—were markedly reduced, reflecting a substantial decline in PSII photochemical performance and overall photosynthetic capacity under heat stress conditions. In the presence of elevated CO₂, both wild type cultivar, Col-0, and *srt2* mutant, SRT2 gene-deficient plants, exhibited a promotion in growth and water status. However, the *srt2* mutant did not show significant improvements in chlorophyll fluorescence parameters (F_v/F_m , F_v/F_o , F_o/F_m) or in the rate of carbon assimilation under heat stress, indicating a limited photosynthetic response. Moreover, the absence of a functional SRT2 gene compromised the efficiency of the electron transport chain and failed to protect the photosynthetic apparatus. Similarly, inhibition of sirtuin activity in Col-0 plants via sirtinol treatment resulted in physiological responses that were either comparable to or lower than those observed in *srt2* mutants under stress conditions. While the induced (SOD and GR) and reduced activities (POX, APX, AsA/DHA and GSH redox state) increased H₂O₂ accumulation, which proved oxidative damage in Col-0 and *srt2*, eCO₂-mediated defense mechanisms differ between Col-0 and *srt2* mutants. The alleviation action of eCO₂ in Col-0 under stress included POX and AsA regeneration (increased APX, MDHAR, DHAR, AsA/DHA). After stress exposure, the elevated CO₂

in *srt2* mutant plants activated POX, AsA and GSH redox status (induced APX, GR, MDHAR, DHAR, AsA/DHA and GSH/GSSG). Collectively, these responses contributed to a significant reduction in lipid peroxidation, highlighting the role of eCO₂ in enhancing reactive oxygen species (ROS) scavenging under stress conditions.

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Author contributions C.O.K., E.Y., and I.T. designed experiments; F.N.A.T. and B.A.A. carried out data analysis; C.O.K., E.Y. and B.A.A. interpreted the results and wrote up the first draft of the manuscript; C.O.K., E.Y. and I.T. critically edited the whole manuscript. All authors read and approved the final manuscript.

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Data Availability Data will be made available on request.

Declarations

Conflict of interest The authors declare no competing interests.

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