









# Foliar application of salicylic acid intensifies antioxidant system and photosynthetic efficiency in tomato plants

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**Received:** Jun. 11, 2021 | **Accepted:** Jan. 11, 2022

**Section Editor:** Mauro Guida dos Santos

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**How to cite:** Aires, E. S., Ferraz, A. K. L., Carvalho, B. L., Teixeira, F. P., Rodrigues, J. D. and Ono, E. O. (20xx). Foliar application of salicylic acid intensifies antioxidant system and photosynthetic efficiency in tomato plants. *Bragantia*, 81, e1522. <https://doi.org/10.1590/1678-4499.20210320>

**ABSTRACT:** Tomatoes are the most important and grown vegetable crop in the world. The salicylic acid (SA) application could improve crop yields due the positive physiological effects of this plant growth regulator. Thus, this study aimed to evaluate the possible effects of SA application on leaf regarding the intensification of antioxidant enzymes activities, chlorophyll *a* fluorescence, gas exchange, and tomato production against environmental stress. This experiment was conducted by the use of Colossal tomato hybrid in a protected environment between July and December 2019. Therefore, a randomized block design with five SA doses was used, as follows: 0; 0.5; 1; 1.5, and 2 mM. Then, applications were performed weekly from 15 to 60 days after transplanted (DAT). At the 45<sup>th</sup> and the 60<sup>th</sup> DAT, the enzymes activities were analysed, such as superoxide dismutase (SOD), catalase activity (CAT) and peroxidase (POD), lipid peroxidation, proline content, chlorophyll *a* fluorescence, gas exchange, and plant height. At the end of the experiment, fruit weight, total and commercial production were also evaluated. Results indicated that foliar application of SA reduced the environmental stress in plants through the intensification of antioxidant system that reduced lipid peroxidation and *qNP* and increased the efficiency of photosystem II and ETR. Furthermore, gas exchange was also influenced by the action of SA in  $g_s$ , favouring *A* and *A/Ci*. The SA dose between 0.5 and 0.8 mM positively enabled the total and commercial production of tomatoes. Therefore, foliar application of SA reduced oxidative damage, and increased photosynthetic efficiency and fruit production.

**Key words:** photosynthesis, production, plant growth regulator, *Solanum lycopersicum*.

## INTRODUCTION

Tomato is the most cultivated vegetable in the world. In 2019, the international tomato production was 180,766,329 million tons on about 5,030,545 hectares; therefore, a yield of 35.9 t·ha<sup>-1</sup> by considering the average between field cultivation and protected environment (FAO 2019). For small and medium scale producers, tomato crops are the only source of income. Nutritionally, tomatoes are the major dietary source of carotenoids such as lycopene; they are also a great source of vitamin C, potassium, and folic acid (Perveen et al. 2015).

In the context of vegetable production, plants are frequently subjected to unfavourable situations for their optimal development and operation caused by alterations in the environment. This set of unfavourable situations is known as environmental stress (Veobides-Amador et al. 2018). Even in protected conditions, tomato crops can face some adversity, for example, high temperature, low relative humidity, pest infestations, and diseases. The response to adversity lead to reactive oxygen species (ROS) generation, that can cause a detrimental effect due to their ability to occasion lipid peroxidation of cellular membranes, protein denaturalization, pigment breakdown, carbohydrate oxidation, DNA damage, and impaired

enzymatic activities (Bose et al. 2014), as also decrease in photosystems efficiency, reduction in the relations of gas exchange and yield loss (Campelo et al. 2015; Costa et al. 2015).

Among the various approaches, the use of plant growth regulators has proven a very effective method of choice to produce climate-resilient crops with prominent yields (Wani et al. 2016). Between the plant growth regulators, one can mention the use of salicylic acid (SA), since it is poorly explored in agriculture when compared to auxins, gibberellins and cytokinin. SA is a phenolic compound that acts on plant growth, ion absorption and substance transport, besides being an important plant signalling molecule associated with their defence mechanism; consequently, increasing plant tolerance to biotic and abiotic stresses (Kazemi 2014; Gorni et al. 2017).

The exogenous application of SA could control the functions of antioxidative enzymes, and it escalates plant tolerance to abiotic stresses (Parashar et al. 2014). Application of SA may mitigate the harmful effects of various stressors, and also mainly focused on higher photosynthetic capacity (Tirani et al. 2013). SA plays a crucial role in plant defence system by modulating antioxidant enzymes and maximizing the net photosynthetic rate (El-Esawi et al. 2017). Application of this regulator activates the enzymes superoxide dismutase, catalase, guaiacol peroxidase, and ascorbate peroxidase and reduces the lipid peroxidation in *Brassica rapa* under drought stress (La et al. 2019). SA pre-treatment could considerably alleviate the harmful impact of heat and high light stress on PSII and it helps to stimulate the rebuilding of photosynthetic function (Zhao et al. 2011). There are many research findings available about individual effects of drought, salinity, high temperatures, and salicylic acid (Jahan et al. 2019; Souri and Tohidloo 2019; Chakma et al. 2021). However, there is no evidence that SA foliar application increases environmental stress tolerance and tomato production under field conditions. It is important in order to increase more productivity of this vegetable, as well as to suggest an alternative for cultivation in regions with adverse conditions for its cultivation.

Based on the arguments presented, our research hypothesized that foliar application of salicylic acid can reduce the harmful effects of environmental stress on tomato plants. To test this hypothesis, we evaluated the chlorophyll *a* fluorescence, gas exchange, antioxidant enzymes activities, lipid peroxidation, proline, and production of tomato plants subjected to SA application.

## MATERIAL AND METHODS

The experiment was conducted in Sao Manuel Experimental Farm of Education, Research and Production (located in the homonymous town) from July to December 2019. This farm belongs to Universidade Estadual Paulista “Júlio de Mesquita Filho” (UNESP), School of Agriculture, Botucatu, SP, Brazil. The geographical coordinates are 22°44'S latitude, and 47°34'W longitude, and it is located at 750 meters above sea level, with a humid subtropical climate. The experiment selected the Colossal tomato hybrid, whose is an Italian-type with determined habit growth, developed by Sakata®. Seedlings were transplanted into 15 kg pots (spacing of 1 m between rows and 0.5 m between plants). When they were about 15 cm tall, they were conducted with the help of bamboo and tape. The protected environment structure was covered with 150 µm low-density polyethylene film with additives, and the sides were closed with a 75% sunblock shade cloth. The average temperature and relative air humidity inside the greenhouse were 27°C and 45% during the experimental period, respectively.

A randomized block design with five treatments and four blocks was used, each plot consisted of four useful plants. The treatments were 0 (control); 0.5; 1; 1.5; and 2 mM of SA, which were applied via foliar spray. In the treatments, the solutions were prepared from 5 mL of SA dissolved in absolute ethanol and supplemented with distilled water and, then, applied weekly for seven weeks; that is, after 15 days of transplantation (DAT) until the 60<sup>th</sup> DAT. The applications were made through a pressurized CO<sub>2</sub> manual sprayer (0.3 kgf per 31 cm<sup>2</sup>) with full conical nozzles; thus, 40 mL of the solution was applied per plant in each application.

Therefore, 15 kg pots were filled with red yellow latosol-sandy phase that was previously collected on the farm itself and presented the following properties: pH (CaCl<sub>2</sub>) = 4.2; M.O. = 9 g·dm<sup>-3</sup>; P(resin) = 2 mg·dm<sup>-3</sup>; H+Al = 31 mmol<sub>c</sub>·dm<sup>-3</sup>; K<sup>+</sup> = 0.6 mmol<sub>c</sub>·dm<sup>-3</sup>; Ca<sup>2+</sup> = 3 mmol<sub>c</sub>·dm<sup>-3</sup>; Mg<sup>2+</sup> = 1 mmol<sub>c</sub>·dm<sup>-3</sup>; CTC = 36 mmol<sub>c</sub>·dm<sup>-3</sup>; and V = 12%. For the surface, drip

irrigation system was conducted with two emitters per pot. Soil correction, fertilization, and fertigation were performed according to the recommendations proposed by Trani et al. (2015).

Furthermore, the photosynthetic parameters related to chlorophyll *a* fluorescence, gas exchange, activity of antioxidant enzymes, lipid peroxidation, proline content, and plant height were recorded at the 45<sup>th</sup> and the 60<sup>th</sup> DAT. The chlorophyll *a* fluorescence was measured using a LI-6400 system with the 6400-40 leaf chamber fluorometer; it was selected fully expanded leaves from the middle third of the plant, which were covered with aluminium that were kept in darkness for 30 min before recording the fluorescence. The minimal fluorescence ( $F_0$ ) was determined under sufficiently low irradiance ( $< 1 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ ). The maximal fluorescence ( $F_m$ ) was determined after a 0.8-s saturation pulse at  $4,200 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$  on the dark-adapted leaves (30 min). In the light-adapted leaves, the radiation of the saturation pulses to determine the maximal fluorescence ( $F_m'$ ) was  $6,000 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$  for 0.8 s, whereas the actinic light was  $200 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ . Measurements of the quantum yield of photosystem (PS) II photochemistry (PSII) were obtained by application of a saturation light pulse ( $6,000 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$  for 0.8 s) under ambient irradiance. The fluorescence parameters, such as variable maximum quantum yield of PSII ( $F_v/F_m$ ), effective quantum yield of photosystem II (PSII) ( $F_v'/F_m'$ ), photochemical quenching ( $qP$ ), non-photochemical quenching ( $qNP$ ), and relative rate of electron transport (ETR), were calculated according to Schreiber et al. (1986).

Both for fluorescence and gas exchange readings were performed in the morning between 9 and 11 a.m. (Ramos et al. 2015). The  $\text{CO}_2$  assimilation rate ( $A$ ,  $\mu\text{mol CO}_2\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ ), transpiration rate ( $E$ ,  $\text{mmol water vapour}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ ), stomatal conductance ( $g_s$ ,  $\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ ), and internal  $\text{CO}_2$  concentration in tomato leaf ( $C_i$ ,  $\mu\text{mol CO}_2\cdot\text{mol}^{-1}\cdot\text{air}$ ) were measured in three plants per treatment with a portable open gas exchange system (LI-6400, LICOR). The  $\text{CO}_2$  concentration entering the leaf cuvette (LCF chamber;  $2 \text{ cm}^2$ , LI-COR) averaged  $400 \mu\text{mol}\cdot\text{mol}^{-1}$ , as provided by the 6400-01  $\text{CO}_2$  mixer (LI-COR). The photosynthetic photon flux density (PPFD) was provided by an artificial light-emitting diode (LED) light source (6400-40 LCF, LI-COR; 90% red and 10% blue spectra), which was set to provide  $1,000 \mu\text{mol photons}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$  in the leaf cuvette, based on curve of light performed previously. The vapor pressure deficit (VPD) inside the leaf cuvette was  $2.08 \pm 0.18 \text{ kPa}$ , which means the relative humidity in the (sample) chamber was  $65.1 \pm 2.3\%$ ; water use efficiency ( $WUE$ ,  $\mu\text{mol CO}_2$  [ $\text{mmol H}_2\text{O}^{-1}$ ]) determined through the relations between  $\text{CO}_2$  assimilation and transpiration rate; and the instant carboxylation efficiency ( $A/C_i$ ) determined through the relation between  $\text{CO}_2$  assimilation rate and the internal  $\text{CO}_2$  concentration of in tomato leaf.

For biochemical analyses, the leaves were collected and frozen instantly in liquid nitrogen. In the laboratory, the leaves were stored in an ultra-freezer at  $-80^\circ\text{C}$  until analysis. Then, the leaves were macerated with the aid of liquid nitrogen until they became powdery, and after that 300 mg were weighed for enzymatic extraction, 200 mg for lipid peroxidation, and 100 mg for proline. The entire process was done with pre-cooled tubes and tweezers to avoid thawing the samples.

The enzymatic extraction was performed according to the methodology proposed by Kar and Mishra (1976), added 5 mL of solution containing 50 mM phosphate buffer (pH 6.7), and 1% (m/v) polyvinylpyrrolidone. The homogenate was centrifuged at  $15,000 \text{ g}$  for 20 min, and the supernatant collected for enzyme assays. To determine the specific activity of antioxidant enzymes, the quantification of soluble proteins of each enzymatic extract was previously performed according to Bradford (1976).

The activity of the antioxidant enzyme superoxide dismutase (SOD) was determined through the ability of the enzyme to inhibit photoreduction of nitroblue tetrazolium (NBT), according to the methodology proposed by Giannopolitis and Ries (1977), with modifications of Dousseau et al. (2016). The absorbance readings were performed at 560 nm (Biotek Epoch<sup>TM</sup> Microplate Elisa Spectrophotometer). One unit of SOD activity was defined as the necessary amount of enzyme to inhibit NBT photoreduction by 50% under assay conditions, expressed in  $\text{U mg}\cdot\text{protein}^{-1}$ .

To determine catalase activity (CAT), we followed the methodology proposed by Havar and McHale (1987), adapted by Dousseau et al. (2016), therefore reducing the absorbance at 240 nm every 30 seconds for 4 minutes by monitoring the hydrogen peroxide consumption via spectrophotometer (Biotek Epoch<sup>TM</sup> Microplate Elisa Spectrophotometer). One unit of CAT activity was defined as the degradation of  $1 \mu\text{M}$  of  $\text{H}_2\text{O}_2$   $\cdot\text{minute}^{-1}$ . For CAT, the molar extinction coefficient ( $\epsilon$ ) was calculated as  $36 \text{ mM}^{-1}\cdot\text{cm}^{-1}$  in  $\text{mKat mg}\cdot\text{protein}^{-1}$ .

The activity of enzyme peroxidase (POD) was determined according to the methodology proposed by Teisseire and Guy (2000) with modifications, and the readings were performed at 430 nm in a spectrophotometer (Biotek Epoch<sup>TM</sup> Microplate

Elisa Spectrophotometer). To calculate POD, it was used the molar extinction coefficient ( $2.5 \text{ mmol}\cdot\text{L}^{-1}\cdot\text{cm}^{-1}$ ) and activity expressed in  $\mu\text{mol}\cdot\text{min}^{-1} \text{ mg}\cdot\text{protein}^{-1}$ .

Lipid peroxidation (MDA) was determined according to the technique proposed by Rama Devi and Prasad (1998). Sample of macerated fresh leaves (200 mg) was homogenized in 5-mL extraction buffer containing 0.25% TBA and 10% TCA and then boiled at  $95^\circ\text{C}$  for 30 min. The reaction mixture was cooled on ice after centrifugated at 10,000 g for 5 min, the absorbance was measured at 560 and 600 nm wavelength, and the results expressed in  $\mu\text{mol}$  of MDA per gram of fresh weight.

The proline content was determined according to the method described by Bates et al. (1973). Sample of macerated fresh leaves (100 mg) was homogenized in 1.5 mL of sulfosalicylic acid 3% and afterwards centrifugated at 10,000 g for 5 min. For colorimetric determinations, a solution of extract, ninhydrin acid, and glacial acetic acid was incubated at  $90^\circ\text{C}$  for 1 h. Then, the reaction was cooled in an iced bath. The chromophore was extracted using 2 mL of toluene and its absorbance at 520 nm. The results were expressed in  $\mu\text{mol}$  of L-proline per gram of fresh weight.

For measuring, tape was used for plant height, the harvest started at the 75<sup>th</sup> DAT and ended at the 120<sup>th</sup> DAT. The fruits of each plot were separated into commercial and non-commercial according to the Tomatoes Standard Classification (CEAGESP 2003) and then counted. Subsequently, the weighing of the fruits was performed with a scale to determine fruit weight, as well as the sum of the production of each harvest, the total and the commercial production of fruits  $\text{kg}\cdot\text{plant}^{-1}$ .

For statistical analysis, data were previously tested for homogeneity through Anderson-Darling test at Minitab program. After testing for normality, analysis of variance (F test) and regression analysis were performed by AgroEstat<sup>®</sup> program. SigmaPlot<sup>®</sup> program helped to create the graphics.

## RESULTS

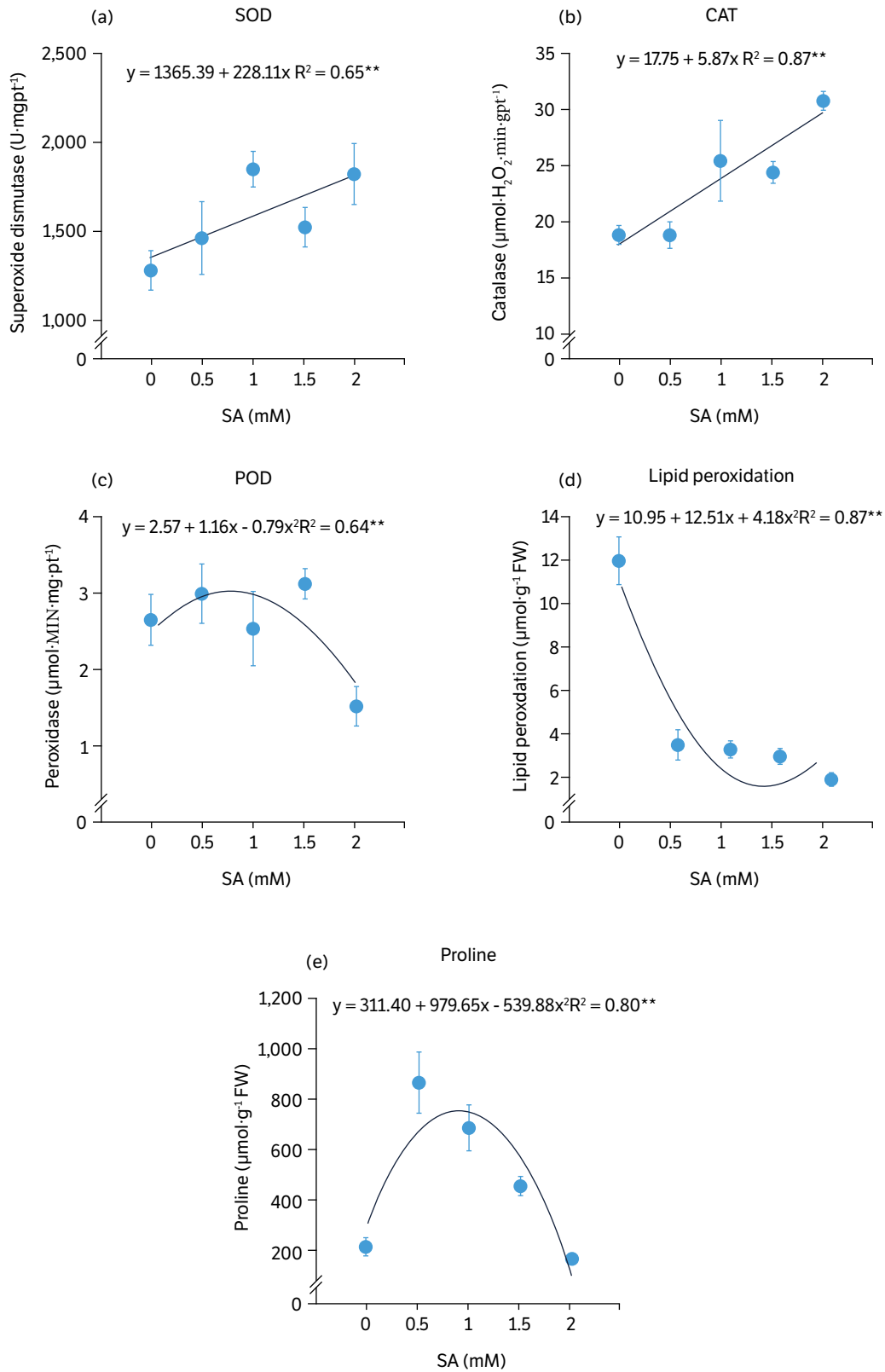
For the activity of antioxidant enzymes SOD, CAT and POD, lipid peroxidation and proline content in tomato leaves treated with SA, a significant difference ( $p < 0.05$ ) was observed in the two evaluations, the 45<sup>th</sup> and the 60<sup>th</sup> DAT. Regarding the chlorophyll *a* fluorescence parameters analysed, there was a significant effect ( $p < 0.05$ ) at the 45<sup>th</sup> DAT for: *qNP*, *ETR* and *Fv/Fm*; and at the 60<sup>th</sup> DAT only *ETR* showed this effect. For gas exchange, all variables were affected ( $p < 0.05$ ) by the application of SA at the 45<sup>th</sup> DAT: *A*, *g<sub>s</sub>*, *Ci*, *E*, *WUE*, and *A/Ci*; at the 60<sup>th</sup> DAT there was a significant effect ( $p < 0.05$ ) for: *A*, *g<sub>s</sub>*, *Ci* and *A/Ci*. Height evaluations at the 45<sup>th</sup> and the 60<sup>th</sup> DAT and production were also significantly affected ( $p < 0.05$ ) by foliar application of SA.

For the activity of the enzymes SOD and CAT, a linear effect was observed according to SA dose (Figs. 1a and 1b), while the activity of POD showed a quadratic effect (Fig. 1c). Such behaviour reduced lipid peroxidation (Fig. 1d) by approximately 70% from 0.5 mM SA at the 45<sup>th</sup> DAT. For the proline content, the application of 0.90 mM SA caused a greater accumulation, that is,  $785 \mu\text{mol}\cdot\text{g}^{-1} \text{ FW}$  at the 45<sup>th</sup> DAT (Fig. 1e).

The analysis of chlorophyll *a* fluorescence is an important factor to identify the possible changes that may occur in photosystem II performance both directly and indirectly. Figure 2 shows significant differences and quadratic equations in the *qNP*, *ETR*, *t* and *Fv/Fm* at the 45<sup>th</sup> DAT. The *qNP* showed lower value at 1.25 mM SA, that is, a 25% reduction in the dissipated energy in a non-photochemical way compared to control (Fig. 2A). The application of 1.0 mM SA caused higher values for *ETR* and *Fv/Fm* (Figs. 2B and 2C).

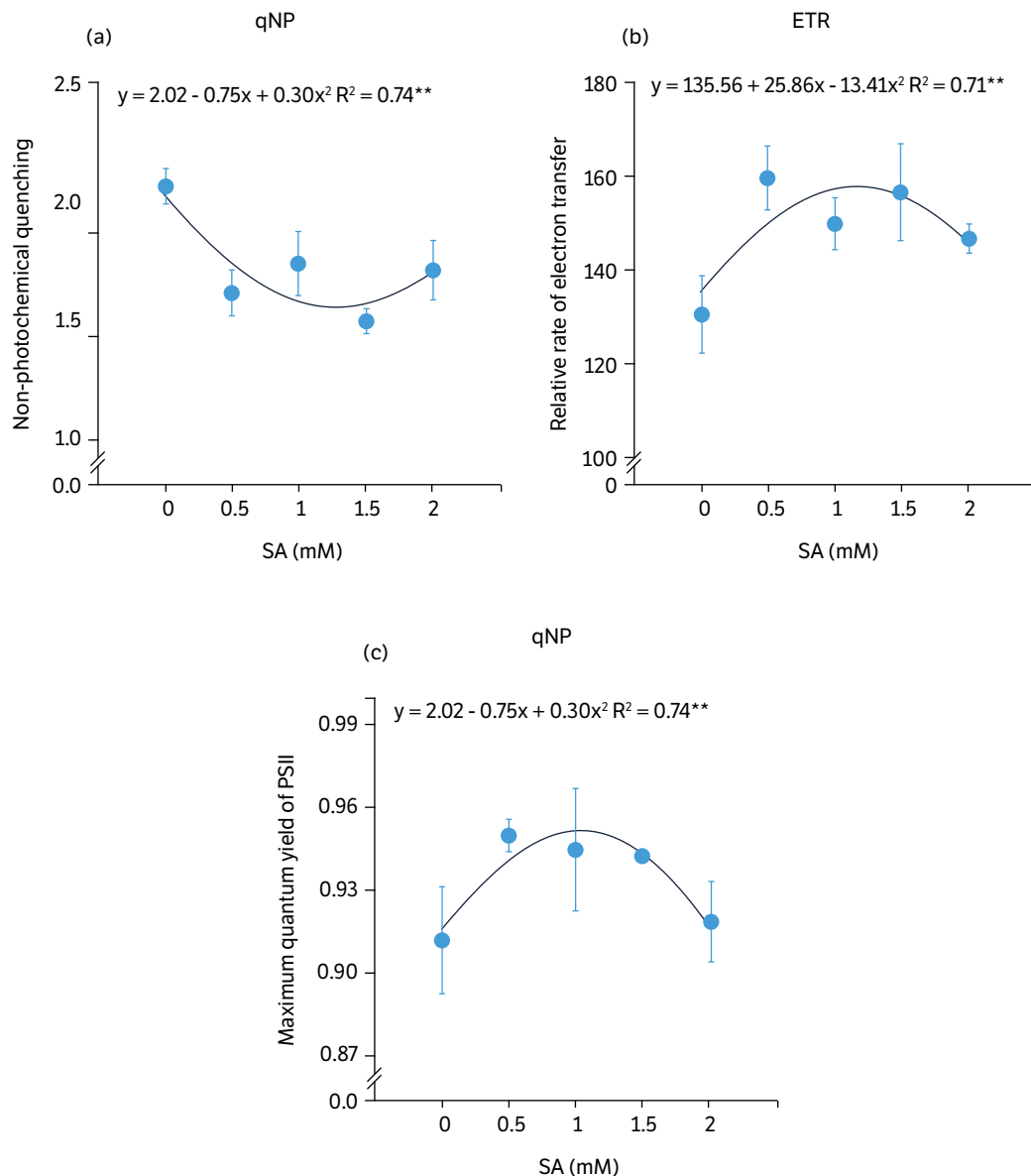
Moreover, the SA application positively influenced all gas exchange variables at the 45<sup>th</sup> DAT, such as *A*, *g<sub>s</sub>*, *E*, *Ci* and *A/Ci* (Fig. 3); thus, following the same trend with increments of 72, 82, 70 and 72%, respectively; compared to control. For *A*, the application of 1.4 mM SA increased to  $17.3 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$  (Fig. 3a), the same dose also increased the rate of stomatal opening to  $0.27 \text{ mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$  (Fig. 3b) and transpiration rate to  $6.54 \text{ mmol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$  (Fig. 3d). At the 45<sup>th</sup> DAT, the *Ci* values (Fig. 3c) dropped from the SA application, and, as a result, it was observed the highest values for *A*, carboxylation efficiency, *A/Ci* at 1.7 mM SA (Fig. 3e).

The activity of the antioxidant enzymes SOD, CAT and POD can be influenced by external factors such as light, water deficit, temperature and even by pest infestations and diseases. In this study, the determining factor that improved the activity of these enzymes was the use of this plant growth regulator. There was an increase in the activity of SOD, CAT and POD



\*\*0.01 < p < 0.05; DAT: days after transplantation.

**Figure 1.** Activity of (a) enzymes superoxide dismutase (SOD), (b) catalase (CAT), (c) peroxidase (POD), (d) lipid peroxidation, and (e) proline content in tomato plants subjected to salicylic acid (SA) application at the 45<sup>th</sup> DAT. Botucatu, SP, 2020. Whiskers represent 95% confidence intervals of means.

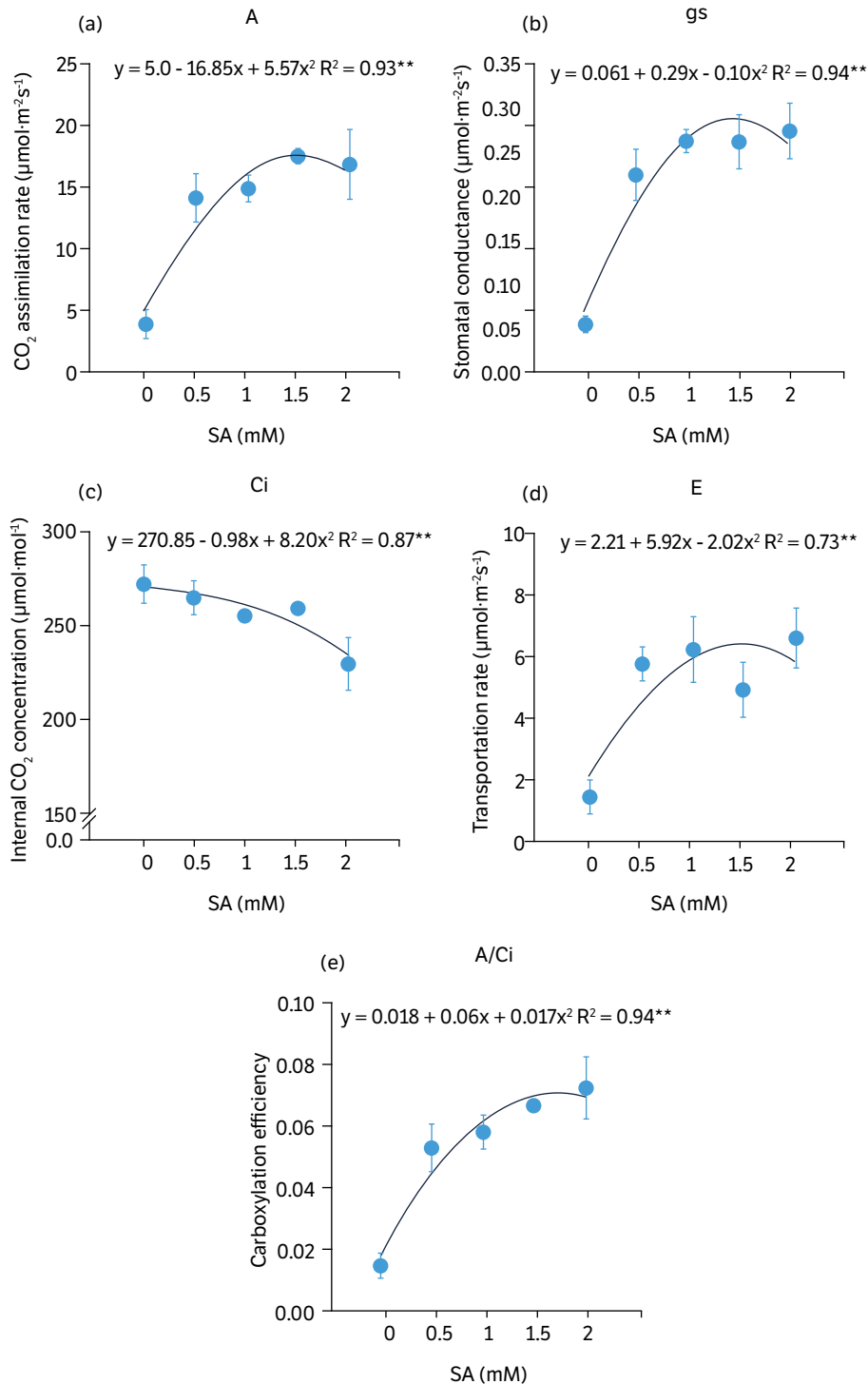


\*\*0.01 < p < 0.05; PSII: effective quantum yield of photosystem II; DAT: days after transplantation.

**Figure 2.** (a) Non-photochemical quenching (*qNP*), (b) relative rate of electron transfer (*ETR*) and (c) maximum quantum yield of PSII (*Fv/Fm*) in tomato plants subjected to salicylic acid (SA) application at the 45<sup>th</sup> DAT. Botucatu, SP, 2020. Whiskers represent 95% confidence intervals of means.

at the 60<sup>th</sup> DAT (Figs. 4a, 4b and 4c) with the dose of 2 mM SA, and, consequently, lipid peroxidation decreased in plants exposed to this treatment (Fig. 4d). The proline content in tomato leaves followed the trend of enzyme activity values, that is, the dose of 2 mM enabled greater accumulation in plants (Fig. 4e).

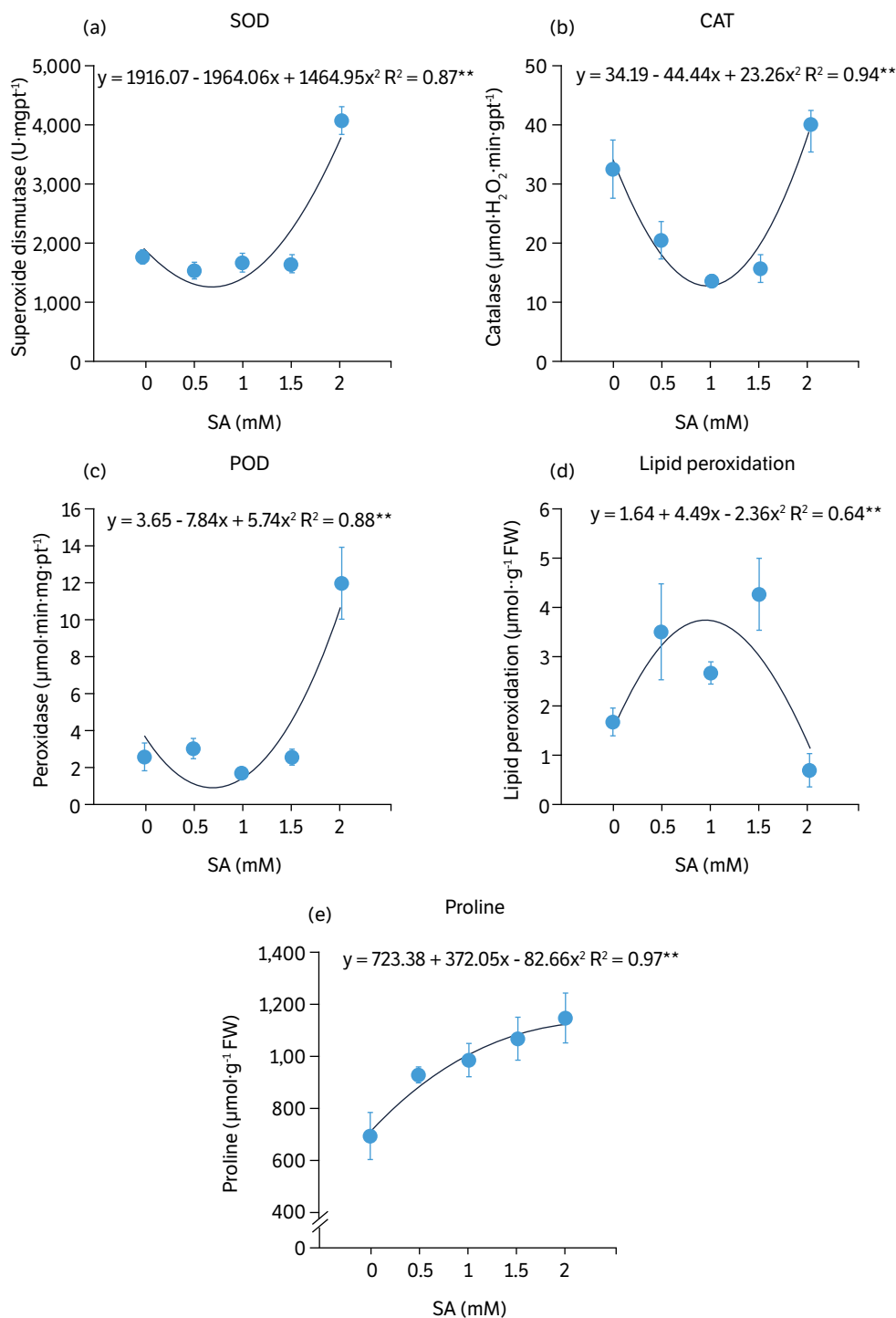
With regards to the analysis of chlorophyll *a* fluorescence, *ETR* was the only one influenced by SA application at the 60<sup>th</sup> DAT (Fig. 5a). The higher *ETR* values directly reflected on higher CO<sub>2</sub> assimilation rate in tomato plants treated with 1.01 and 1.4 mM SA, respectively (Fig. 5b). Most gas exchange variables were positively influenced by the action of the exogenous application of SA at the 60<sup>th</sup> DAT, except transpiration rate. Similar behaviour happened in the evaluations at the 45<sup>th</sup> DAT; thus, revealing the ability of this plant growth regulator to improve the physiological characteristics of tomato plants, when the applications were repeated at frequent intervals. The *g<sub>s</sub>* followed the same behaviour as *A* with a quadratic tendency;



\*\* $0.01 < p < 0.05$ ; PSII: effective quantum yield of photosystem II; DAT: days after transplantation.

**Figure 3.** (a)  $CO_2$  assimilation rate ( $A$ ), (b) stomatal conductance ( $g_s$ ), (c) internal  $CO_2$  concentration in the leaf ( $C_i$ ), (d) transpiration rate ( $E$ ), and (e) carboxylation efficiency ( $A/C_i$ ) in tomato plants subjected to salicylic acid (SA) application at the 45<sup>th</sup> DAT. Botucatu, SP, 2020. Whiskers represent 95% confidence intervals of means.

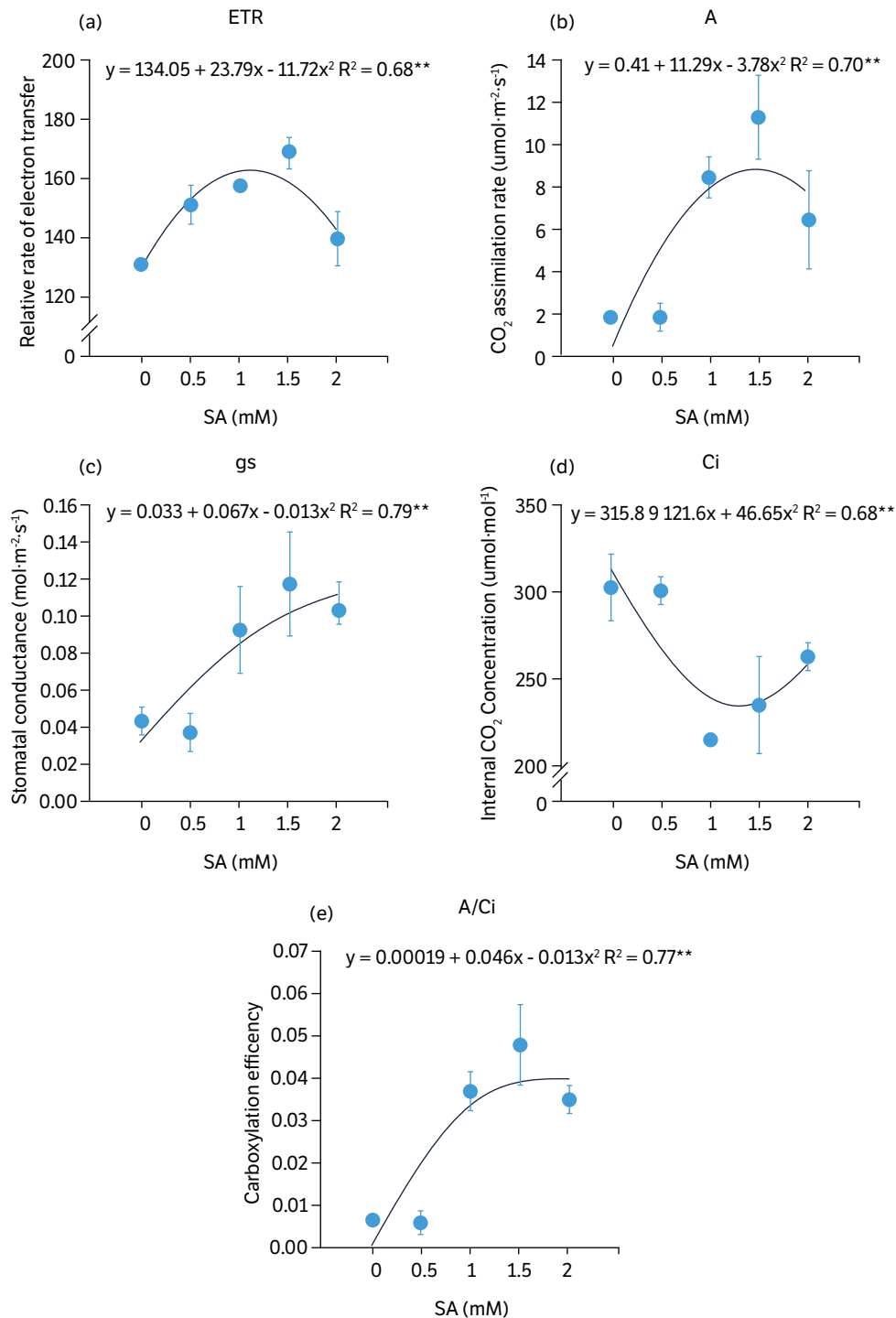
and 2 mM SA promoted greater stomatal opening to  $0.1193 \text{ mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$  (Fig. 5c), while  $C_i$  decreased in plants treated at 1.31 mM SA, because of the great action of the enzyme ribulose 1,5-bisphosphate carboxylase/oxygenase (Rubisco) that could have led to a great carboxylation efficiency in plants (Figs. 5d and 5e).



\*\*0.01 < p < 0.05; DAT: days after transplantation.

**Figure 4.** (a) Activity of enzymes superoxide dismutase (SOD), (b) catalase (CAT), (c) peroxidase (POD), (d) lipid peroxidation, and (e) proline content in tomato plants subjected to salicylic acid (SA) application at the 60<sup>th</sup> DAT. Botucatu, SP, 2020. Whiskers represent 95% confidence intervals of means.

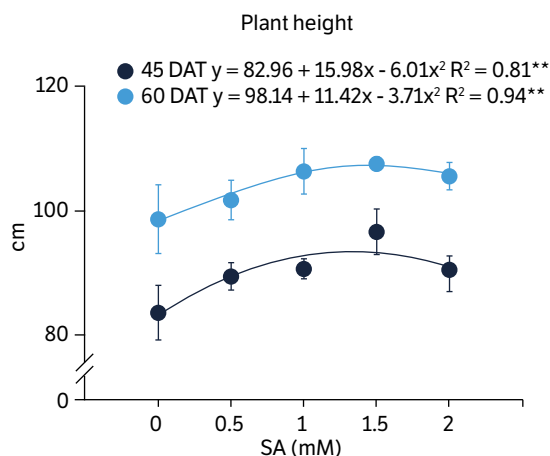
The SA application in tomato has positively modified both physiological and biochemical characteristics of the plants; consequently, impacting on the vegetative growth. There was a significant difference for plant height at the 45<sup>th</sup> and the 60<sup>th</sup> DAT (Fig. 6). In the first evaluation, the highest height (93 cm) was observed in plants submitted to 1.32 mM SA, while 1.53 mM SA increased the plant height to 106.9 cm in the second evaluation. When SA was applied in the beginning of



\*\*0.01 < p < 0.05; DAT: days after transplantation.

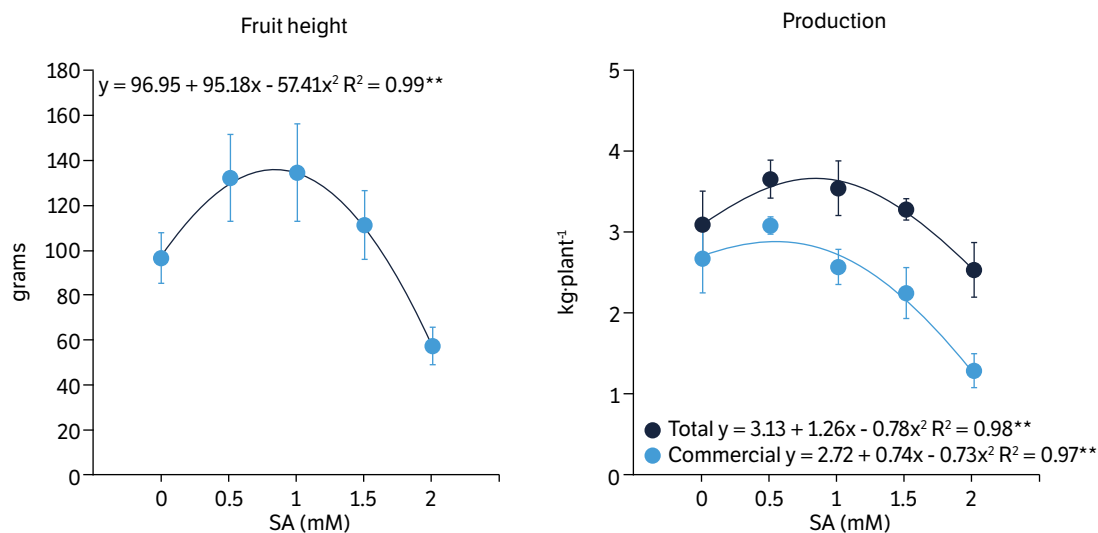
**Figure 5.** (a) Relative rate of electron transfer (ETR), (b)  $CO_2$  assimilation rate (A), (c) stomatal conductance ( $g_s$ ), (d) internal  $CO_2$  concentration in the leaf (Ci), and (e) transpiration and carboxylation efficiency (A/Ci) in tomato plants subjected to salicylic acid (SA) application at the 60<sup>th</sup> DAT. Botucatu, SP, 2020. Whiskers represent 95% confidence intervals of means.

the experiment, it improved the final fruit production, because of the increase in the fruit weight to 136 g at 0.82 mM SA (Fig. 7a). Therefore, the total and commercial fruit production also increased with SA applications, as both are influenced by the fruit weight. Thus, results indicated a total fruit production of 3.63 kg·plant<sup>-1</sup> at 0.80 mM SA and a commercial fruit production of 2.90 kg·plant<sup>-1</sup> at 0.5 mM SA (Fig. 7b).



$^{**}0.01 < p < 0.05$ ; DAT: days after transplantation.

**Figure 6.** Plant height in tomatoes plants subjected to salicylic acid (SA) application at the 45<sup>th</sup> and the 60<sup>th</sup> DAT. Botucatu, SP, 2020. Whiskers represent 95% confidence intervals of means.



$^{**}0.01 < p < 0.05$ .

**Figure 7.** Fruit weight, total and commercial production of tomatoes fruits in plants subjected to salicylic acid (SA) application. Botucatu, SP, 2020. Whiskers represent 95% confidence intervals of means.

## DISCUSSION

Our results suggested that foliar application of SA intensifies the plant's antioxidant system, activating the enzymes SOD, CAT, POD and, consequently, reducing lipid peroxidation. With the maintenance of cell membrane integrity, the photosynthetic apparatus of plants is protected, which increases the efficiency of PSII in plants treated with SA. This plant regulator also increases the accumulation of proline, an important osmoregulator, ensuring high  $g_s$  and  $A$  and increasing the photoassimilates. The tomato plants treated with SA also presented greater height and fruit production.

In this study, we sought to understand how the exogenous application of SA occurs on the physiological and productive mechanisms in tomato plants. The SA application in the beginning of the vegetative phase was chosen to reduce environmental stress and increase production through floral induction. Some studies have already showed that SA action could be used as

a flowering inducer, as well as the ability to increase the number of flowers per plant in papaya (Martín-Mex et al. 2012), and petunia (Martín-Mex et al. 2010). It was not observed the influence of SA application on the improvement in the number of flowers and fruits or the anticipation of harvest in tomato plants, even though the plant growth regulator (SA) increased fruit weight and, consequently, the production per plant.

It was noticed that the mechanism of action of SA is not well understood, mainly because it can differ from species to species, as well as it varies according to environmental conditions and applied dose. High doses of this plant regulator can cause high levels of oxidative stress, leading to reduced stress tolerance. Both plant growth and drought tolerance of wheat plants are reduced when a high dose of SA (2-3 mM) is applied, while plant growth is increased by application of a low concentration, around 0.5 mM (Kang et al. 2012). Responses of greater CO<sub>2</sub> assimilation, increase in stomatal conductance and, consequently, increase in dry matter of barley plants are reported by Habibi (2012) when applying a dose of 500 µM of SA under water-limited conditions. Foliar application of SA (1 mM) strengthened the antioxidant system of maize plants, thus making the cultivar studied more tolerant to water deficit (Saruhan et al. 2012). As stated, the dose of SA to be used can vary according to the species and environmental condition. For this reason, the present research used the doses of 0.5-2 mM in order to increase the spectrum of concentrations tested for the cultivation condition protected from tomato.

Since the SA responds as an elicitor in plants, SA can generate several effects that will depend on each species, plant development phase, dosage, and exposure time to treatment (Akbulut et al. 2014). In this study, the foliar application of SA was an important precursor for the accumulation of biochemical compounds, such as proline. Besides that, SA was also crucial to activate the antioxidant enzymes SOD, CAT, and POD at the 45<sup>th</sup> and the 60<sup>th</sup> DAT. Nevertheless, oxygen-dependent metabolic processes, such as aerobic respiration, photosynthesis, and photorespiration lead to the production of ROS (Foyer and Noctor 2003). Thus, plants are subject to biotic or abiotic stresses even under favourable crop conditions, that is why tomato plants produced ROS, which were reduced, as well as lipid peroxidation in plants treated with SA.

Similar results were obtained in corn (Ahmad et al. 2017) and potato (Faried et al. 2017). These authors also reported the SA ability in the parity between ROS generation and detoxification capacity through the response of activation and accumulation of the following enzymes SOD, CAT, POD and osmoregulators, such as proline (Sharma et al. 2014). By associating both chlorophyll *a* fluorescence and the activity of antioxidant enzymes, the control treatment showed greater *qNP*, that is, the excess of energy was dissipated in the form of fluorescence and heat, leading to a possible increase in the generation of ROS. Thus, less activity of the enzymes SOD, CAT and POD may have caused more lipid peroxidation. However, the SA application was efficient for reducing *qNP*, which may have led to the production of ROS even under low *qNP* values; therefore, these were reduced by the action of antioxidant enzymes. The process of chlorophyll excitation by light induces the formation of ATP and NADPH<sub>2</sub>, and these products, in turn, are consumed in the Calvin-Benson cycle, by reactions catalysed by enzymes that reduce atmospheric CO<sub>2</sub> into phosphate trioses (Taiz et al. 2017). Therefore, the foliar application of AS affected the light and carboxylation reactions of photosynthesis, allowing the tomato plant to obtain greater growth and productivity.

Regarding to the fluorescence, the *Fv/Fm* was higher in plants with SA treatment, reinforcing that SA increases the photosynthetic efficiency of tomato. This variable can be correlated with the photosynthetic efficiency from the leaf and a sensitive indicator of the plant photosynthetic performance (Krause and Weis 1991). The *Fv/Fm* reduction can be an indicative of photoinhibitory damage caused by the incidence of photon flux density, when plants are subjected to a wide range of environmental stresses (Björkman and Demmig 1987). As the *Fv/Fm* values observed in tomato plants treated with SA (0.5 and 1 mM) were higher than 0.95, it was possible to verify that these plants have overcome environmental stress, leading to the activation of the antioxidant system, so as not to compromise their physiological performance. This system may also have contributed to maintaining the integrity of chloroplast membranes, responsible for photosynthesis light reactions (Taiz et al. 2017).

Plants that did not receive SA were under environmental stress, because of the reduction of *Fv/Fm*, that is, the SA application reduced this type of stress and increased the photosynthetic efficiency of tomato plants. Similar results have also been reported in citrus (Khoshbakht and Asgharei 2015). *ETR* tracks the results of *Fv/Fm*, in which untreated plants suffered environmental stress and, eventually, blocked the electrons transfer. Since *ETR* is an important parameter to verify the integrity of photosystems, as it is indicative of the ability to transfer electrons between PSII and PSI for the generation

of NADPH<sub>2</sub> and ATP. The role of exogenous SA on the characteristics of chlorophyll *a* fluorescence in tomato plants is the maintenance of the integrity of the photosynthetic apparatus by raising *Fv/Fm* and *ETR*, in time, minimizing the excess of effect of excitation energy on the PSII; thus, protecting the photosystem components from the deleterious effects of high *qNP* values.

The foliar application of SA increased the activity of antioxidant enzymes and, in time, reduced lipid peroxidation. The photosynthetic apparatus of the treated plants was protected with ROS elimination; thus, leading to an increase in *A* in tomato plants. Similar results caused by SA application and the protection and improvement of the photosynthetic apparatus have also been reported in soybeans (Li et al. 2020). The increase in *g<sub>s</sub>* promoted by the SA allowed greater availability of CO<sub>2</sub> in leaf mesophyll cells; thus, increasing *A*, *E* and *A/Ci*. However, *Ci* was reduced, while *g<sub>s</sub>* increased. This may be an indication that the increase in *A* occurs due to the higher *g<sub>s</sub>* in plants treated with SA. This behaviour has also been described in *Brassica juncea* (Parashar et al. 2014). The higher *A/Ci* values observed in plants treated with SA demonstrates the greater activity of enzyme rubisco in fixing CO<sub>2</sub> during the photosynthetic process, related to the greater *g<sub>s</sub>* that allowed a higher *A* value.

The relationship between the application of SA and *g<sub>s</sub>* can be explained by the interaction between this regulator and abscisic acid (ABA). In plants, ABA works as a major regulatory component to govern stomatal movement in response to drought and others stress (Schroeder and Keller 1992; Sehar et al. 2021) and stimulates stomatal closure via second messengers such as ROS, nitric oxide, calcium, and protein kinases (Negi et al. 2008). The ROS generated by environmental stress were reduced by SA, which may have reduced the ABA signalling for stomatal closure, resulting in increased *g<sub>s</sub>* in tomato plants treated with SA.

Even if treatment increases *g<sub>s</sub>* and *A*, the plant depends on internal CO<sub>2</sub> for organic synthesis. Thus, the foliar application of SA increased *A* and reduced *Ci*, resulting in superior organic synthesis. This relation was also observed in *Mentha x piperita* L., in which SA application increased the carboxylation efficiency (Gonçalves et al. 2020). These same authors reported that SA influenced the primary metabolism, increasing gas exchange and biomass production in this species. Greater organic synthesis can result in increased growth potential, dry matter accumulation and final product quality. In this context, a foliar application of SA resulted in greater organic synthesis, and, most importantly, plants that received this regulator were able to translocate the assimilated carbon for growth and fruit production.

The increase in plant growth caused by the foliar application of SA may be related to the protective role of this plant growth regulator under the cell membrane, in addition to greater CO<sub>2</sub> assimilation and carboxylation efficiency. Another possible association between SA application and increased growth is the ability of this plant growth regulator to prevent the degradation of indolylacetic acid and cytokinin, which are two important plant hormones in cell division and elongation (Shakirova et al. 2003). Wheat plants treated with exogenous application of SA were subjected to salinity stress and, therefore, showed the same result (Shakirova et al. 2003). Downes e Crowell (1998) also proposed that SA action inhibits the activity of the enzyme 1-aminocyclopropane-1-carboxylic acid (ACC) synthase, preventing ethylene synthesis, that is, a plant hormone that inhibits plant stem elongation.

Positive outcomes related to the SA application were also observed in fruit weight and tomato production. Consequently, the application of this plant growth regulator can be a viable alternative for management practices of this crop. The SA application promoted an increase in fruit size and cucumber production (Preciado-Rangel et al. 2019). Preciado-Rangel et al. (2019) considered that SA acted as a growth promoter, which accelerated cell division and increased fruit size and yield. The doses of 0.5 and 0.8 mM were more efficient for commercial and total production; but these doses were also lower than those that promoted higher activity for antioxidant enzymes, chlorophyll *a* fluorescence, and gas exchange. Therefore, doses greater than 1.2 mM decreased production. Probably, high concentrations of this plant growth regulator can cause a hormonal imbalance, which can inhibit cell division and weight gain by fruits.

The higher production observed in plants treated with SA can be associated with chlorophyll *a* fluorescence and gas exchange responses. In summary, the exogenous foliar application of SA in tomato plants acted as an elicitor, activating the enzymes SOD, CAT and POD, that neutralized ROS and reduced lipid peroxidation; consequently, protecting the photosynthetic apparatus of the plants. Moreover, this protection was observed through the greater efficiency of photosystem II, *A* and *A/Ci*.

Finally, plants treated with SA presented the highest amount of assimilated carbon and, then, this photoassimilation was translocated to the fruit, that lead the greater weight and yield.

## CONCLUSION

The SA application in tomato plants minimized the effects of environmental stress by increasing the activity of the enzymes SOD, CAT and POD and reducing lipid peroxidation, protecting the photosynthetic apparatus, ensuring the proper functionality of the PSII. Also, it improved,  $g_s$  which increased  $A$ , that is, greater relation of  $A/C_i$ ; consequently, reflecting on plant height and weight accumulation in fruits.

## AUTHORS' CONTRIBUTION

**Conceptualization:** Rodrigues, J. D. and Ono, E. O.; **Methodology:** Aires, E. S., Ferraz, A. K. L., Carvalho, B. L. and Teixeira, F. P.; **Investigation:** Aires, E. S., Ferraz, A. K. L., Carvalho, B. L. and Teixeira, F. P.; **Project administration:** Aires, E. S.; **Data curation:** Aires, E. S.; **Formal analysis:** Aires, E. S., Ferraz, A. K. L., Carvalho, B. L. and Teixeira, F. P.; **Writing – original draft:** Aires, E. S. **Supervision:** Rodrigues, J. D. and Ono, E. O.; **Writing – review and editing:** Rodrigues, J. D. and Ono, E. O.; **Validation:** Rodrigues, J. D. and Ono, E. O.; **Visualization:** Rodrigues, J. D. and Ono, E. O.

## DATA AVAILABILITY STATEMENT

All dataset were generated and analysed in the current study.

## FUNDING

Coordenação de Aperfeiçoamento de Pessoal de Nível Superior  
[<https://doi.org/10.13039/501100002322>]  
Financial Code 001.

## ACKNOWLEDGMENTS

The authors would like to express their gratitude to the Faculdade de Ciências Agronômicas of Universidade Estadual Paulista “Júlio de Mesquita Filho” (UNESP), Botucatu campus, and all its servers, who contributed to the development of this study.

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