UNIVERSIDADE ESTADUAL PAULISTA
"JÚLIO DE MESQUITA FILHO"
Campus de Botucatu

FACULDADE DE CIÊNCIAS AGRONÔMICAS
UNESP | BOTUCATU

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ÁCIDO SALICÍLICO NA MITIGAÇÃO DE ESTRESSES EM TOMATEIRO

Botucatu

2022

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Tese apresentada à Faculdade de Ciências Agronômicas da Unesp Câmpus de Botucatu, para obtenção do título de Doutor em Agronomia: Horticultura.

Orientadora: Profa. Dra. Elizabeth Orika Ono

Botucatu

2022

A298a

Aires, Eduardo Santana
Ácido salicílico na mitigação de estresses em tomateiro / Eduardo Santana Aires. -- Botucatu, 2022
73 p. : il., tabs., fotos

Tese (doutorado) - Universidade Estadual Paulista (Unesp), Faculdade de Ciências Agronômicas, Botucatu
Orientadora: Elizabeth Orika Ono

1. Estresse abiótico. 2. Fotossíntese. 3. Regulador vegetal. 4. Solanum lycopersicum L.. I. Título.

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TÍTULO DA TESE: ÁCIDO SALICÍLICO NA MITIGAÇÃO DE ESTRESSES EM TOMATEIRO

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Botucatu, 25 de novembro de 2022

Aos meus pais Laerte e Dalva,
Aos meus irmãos Laerte (*in memorian*) e Claudia,
Aos meus sobrinhos João e Cecília,
Dedico

AGRADECIMENTOS

À Deus, pela dádiva da vida.

Aos meus familiares Laerte, Dalva, Laerte (*in memorian*) e Claudia por todo apoio em todos os momentos.

À minha orientadora Elizabeth Orika Ono, que carinhosamente chamamos de Beth, pela recepção, amizade, compreensão e todos os conhecimentos transmitidos.

Ao prof. João Domingos (Mingo) pela amizade, atenção, companheirismo e transmissão de valiosos conhecimentos.

Ao prof. Fernando Putti pela amizade e auxílio nas análises estatísticas.

À técnica Maria Regina Moretto do Laboratório de ELISA – Unipex pela disponibilidade e auxílio nas análises bioquímicas.

Aos amigos Andrew Kim e Fabricio Palla por me receberem no grupo de pesquisa, por toda ajuda em campo e laboratório, e, principalmente, pela amizade e parceria.

Às companheiras e amigas de laboratório Beatriz Lívero e Isabelly Marques.

Aos amigos da pós-graduação: Politon, Nicholas, Camila, Thatiane, Jorgiani, Laura, Rafaelly, Patrícia Versuti.

Aos funcionários da Fazenda Experimental São Manuel, em especial Rocha e Daniel, por toda ajuda e amizade.

Às funcionárias do Departamento de Botânica Inara e Heloísa por toda ajuda e disponibilidade.

Aos funcionários do Departamento de Horticultura Edivaldo, Sandra e Eduardo pelas conversas, ajuda e compreensão.

À Faculdade de Ciências Agronômicas – FCA.

Ao Conselho do Programa de Pós-Graduação em Agronomia (Horticultura), em nome do prof. Marco Tecchio pelo apoio e ajuda durante o curso de doutorado.

O presente trabalho foi realizado com apoio da Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) - Código de Financiamento 001.

RESUMO

O objetivo deste estudo foi avaliar as respostas fisiológicas, bioquímicas e produtivas provocadas pela aplicação foliar de ácido salicílico (AS) em plantas de tomateiro. Assim, dividimos essa pesquisa em dois capítulos; com capítulo I investigamos a resposta de tomateiro cultivado em ambiente protegido e submetido à aplicação de doses de AS. O delineamento experimental utilizado foi em blocos ao acaso (DBC), com cinco tratamentos: 0,0; 0,5; 1,0; 1,5 e 2,0 mM de AS e quatro repetições. As aplicações foram realizadas semanalmente dos 15 aos 60 dias após o transplantio (DAT). Analisou-se aos 45 e 60 DAT a atividade das enzimas SOD, CAT e POD, peroxidação lipídica, prolina, fluorescência da clorofila *a*, trocas gasosas e altura das plantas. Ao final do experimento computou-se a produção total, comercial e peso dos frutos. A aplicação foliar de AS reduziu o estresse ambiental, através da ativação de enzimas antioxidantes que reduziram a peroxidação lipídica, também reduziu o *qNP* e aumentou a eficiência do fotossistema II e ETR. As tocas gasosas também foram melhoradas pela ação do AS amentando a g_s, que favoreceu *A* e *A*/*Ci*. Por fim, a dose entre 0,5 e 0,8 mM de AS favoreceu a produção total e comercial do tomateiro. Assim, a aplicação foliar de AS reduz estresse oxidativo e aumenta a eficiência fotossintética e produtiva em plantas de tomate. No capítulo II, objetivou-se avaliar o efeito mitigatório do AS em tomateiro sob défice hídrico, as aplicações das doses 0,0; 0,5; 1,0; 1,5 e 2,0 mM de AS foram realizadas semanalmente dos 15 aos 60 DAT, avaliamos a assimilação de CO_2 (*A*), condutância estomática (g_s), transpiração (*E*), eficiência no uso da água (*EUA*) e eficiência de carboxilação (*A*/*Ci*) aos 15, 30 e 45 dias de défice hídrico continuo, como também, a produtividade de plantas de tomateiro em ambiente protegido, em dois anos consecutivos (2019 e 2020). Os resultados mostram que o défice hídrico reduziu a *A*, g_s, *E* e *A*/*Ci* das plantas de tomateiro e a aplicação foliar de AS aumentou esses parâmetros em todas as épocas de avaliação, resultando em valores semelhantes ou até maiores que em plantas sem restrição hídrica. Além disso, o estresse hídrico causou aborto floral nas plantas de tomateiro, sem a aplicação de AS, reduzindo o número de frutos e produção total e comercial. Em contrapartida, plantas que receberam cerca de 1.3 mM de AS aumentaram a *A* e *A*/*Ci* e translocaram os fotoassimilados, principalmente, para as flores e frutos, reduzindo o aborto floral e aumentando a produção total e comercial de frutos. Assim, a aplicação foliar de AS é eficiente na mitigação dos efeitos deletérios do défice hídrico em tomateiro sobre as trocas gasosas e produção. A aplicação foliar desse regulador vegetal pode ser indicada para condições de campo em regiões com baixa disponibilidade hídrica ou em supressão hídrica por motivos climáticos.

Palavras-chave: estresse abiótico; fotossíntese; regulador vegetal; *Solanum lycopersicum* L.

ABSTRACT

The objective of this work was to evaluate the physiological, biochemical and productive responses caused by the foliar application of salicylic acid (SA) in tomato plants. Thus, this research was divided into two chapters; with chapter I, the response of tomato plants grown in a protected environment and submitted to the application of doses of SA was investigated. The experimental design used was in randomized blocks, with five treatments: 0.0; 0.5; 1.0; 1.5 and 2.0 mM AS and four replicates. Applications were made weekly from 15 to 60 DAT. At 45 and 60 DAT, the activity of SOD, CAT and POD enzymes, lipid peroxidation, proline, chlorophyll *a* fluorescence, gas exchange and plant height were analyzed. At the end of the experiment, the total and commercial production and fruit weight were calculated. Foliar application of SA reduced environmental stress, through the activation of antioxidant enzymes that reduced lipid peroxidation, also reduced *qNP* and increased the efficiency of photosystem II and *ETR*. The gas burrows were also improved by the action of SA on *gs*, which favored *A* and *A*/*Ci*. Finally, the dose between 0.5 and 0.8 mM of SA favored the total and commercial production of tomato. Thus, foliar application of SA reduces oxidative stress and increases photosynthetic and productive efficiency in tomato plants. In chapter II, the objective was to evaluate the mitigating effect of AS on tomato under water deficit, the applications of doses 0.0; 0.5; 1.0; 1.5 and 2.0 mM SA performed weekly from 15 to 60 DAT, we evaluated the assimilation of CO_2 (*A*), stomatal conductance (g_s), transpiration (*E*), water use efficiency (*WUE*) and carboxylation (*A*/*Ci*) at 15, 30 and 45 days of continuous water deficit, as well as the productivity of tomato plants in a protected environment, in two consecutive years (2019 and 2020). The results show that the water deficit reduced the *A*, g_s, *E* and *A*/*Ci* of tomato plants and the foliar application of SA increased these parameters in all evaluation times, resulting in similar or even higher values than in plants without restriction water. In addition, water stress caused floral abortion in tomato plants, without the application of SA, reducing the number of fruits and total and commercial production. In contrast, plants that received about 1.3 mM of SA increased *A* and *A*/*Ci* and translocated the photoassimilates, mainly to flowers and fruits, reducing floral abortion and increasing total and commercial fruit production. Thus, foliar application of SA is efficient in mitigating the deleterious effects of water deficit in tomato on gas exchange and production. The foliar application of this plant regulator can be indicated for field conditions in regions with low water availability or in water suppression for climatic reasons.

Keywords: abiotic stress; photosynthesis; plant growth regulator; *Solanum lycopersicum* L.

SUMÁRIO

INTRODUÇÃO GERAL

A produção agrícola e a sua viabilidade econômica estão diretamente ligadas aos fatores climáticos, e sujeitas às restrições ambientais na forma de estresses abióticos. Entre as limitações abióticas, o estresse hídrico por falta d'agua é o fator mais limitante que implica na redução da fotossíntese, pois causa danos no cloroplasto e inativa a transferência de elétrons, que por sua vez, resulta na formação de espécies reativas de oxigênio, acarretando em danos foto-oxidativos (SHARMA et al., 2012). Dessa forma, compreender os efeitos do défice hídrico sobre o metabolismo das plantas e os mecanismos de tolerância à seca em diversas culturas, possibilitará satisfatório desempenho agronômico das mesmas (BEEBE et al., 2013). Em tomateiro, a restrição hídrica reduz o crescimento vegetativo (PERVEZ et al., 2009) radicular, condutância estomática e fotossíntese (JANGID et al., 2016). A redução da ETc em 60% causou decréscimo na altura da planta e área foliar e pigmentos fotossintéticos em tomateiro (NANGARE et al., 2016), demonstrando a sensibilidade dessa cultura a restrição de água.

O défice hídrico é o fator ambiental adverso mais comum que, por sua vez, reduz seriamente a produtividade das culturas. Um dos mecanismos dos vegetais para contornar a situação de estresse hídrico, afim de se manter um suprimento adequado de água dentro da planta é o desenvolvimento de raízes longas para alcançar a umidade profunda do solo (XIONG et al., 2006) ou reduzir a perda de água por transpiração (CHASTAIN et al., 2014). Assim, os estômatos desempenham papel importante na adaptação das plantas ao estresse hídrico. Dessa forma, a tolerância à seca refere-se à capacidade de uma planta em suportar a perda de água e continuar a se desenvolver quando as condições de umidade retornam (MIURA e TADA, 2014).

Outras formas de respostas das plantas ao estresse hídrico abrangem muitos genes, processos bioquímicos e moleculares, como: detecção, percepção e transdução de sinais, controle transcripcional, a ativação de mecanismos de pronta resposta ao estresse, entre os quais a desintoxicação de espécies reativas de oxigênio (ERO) por enzimas antioxidantes (como a superóxido dismutase, a catalase e a ascorbato peroxidase) e a osmoproteção por solutos compatíveis e removedores de radicais livres (como a glutationa e a prolina) (LISAR et al., 2012).

Entretanto, o estresse na planta pode ser diminuído por meio da aplicação de eliciadores, que mediam as reações de defesa da planta. Dentre os principais eliciadores destacam-se os ácidos orgânicos, tais como os ácido salicílico, ascórbico e cítrico. O ácido salicílico (AS) está presente na maioria das plantas com várias funções reguladoras no metabolismo vegetal (HAYAT et al., 2010; KANG et al., 2014). Este ácido exerce, também, papel importante na regulação de vários processos fisiológicos na planta (SHI et al., 2005), sendo que o acúmulo de AS é um importante componente nos sinais de tradução das principais vias para a resistência sistêmica adquirida (SONG et al., 2004). Contudo, o mecanismo exato de ação do AS não é bem compreendido, principalmente, porque este pode diferir de espécie para espécie, bem como pode variar de acordo com as condições ambientais (PÁL et al., 2014).

Trabalhos já indicam algumas respostas das plantas sob défice hídrico quando aplicado o ácido salicílico (AS). Doses elevadas desse regulador vegetal podem causar níveis elevados de estresse oxidativo, levando à redução de tolerância ao estresse. Tanto o crescimento das plantas quanto a tolerância à seca de plantas de trigo são reduzidos quando aplicada elevada dose de AS (2-3 mM), enquanto que o crescimento das plantas é aumentado pela aplicação de baixa concentração, em torno, de 0,5 mM (KANG et al., 2012). Respostas de maior assimilação de CO_2, aumento na condutância estomática e, consequentemente, acréscimo na matéria seca de plantas de cevada, são reportados por Habibi (2012) quando aplicada dose de 500 µM de AS em condição de limitação hídrica. A aplicação foliar de AS (1,0 mM) fortaleceu o sistema antioxidante de plantas de milho, tornando, assim, a espécie estudada mais tolerante ao défice hídrico (SARUHAN et al., 2012). Pál et al. (2014), avaliando genótipos de arroz, submetidos à aplicação de AS em diferentes níveis de tolerância à seca, relataram que o efeito da aplicação exógena de AS em relação à aquisição de tolerância ao estresse nas plantas nem sempre é óbvio, pois depende de uma gama de fatores.

Levando em consideração que o mecanismo de ação do AS pode diferir entre espécies vegetais, avaliar esse efeito em culturas de importância econômica pode aumentar a produtividade e mitigar estresses abióticos. O tomateiro (*Solanum lycopersicum* L.) está dentre as hortaliças de grande importância, pois é a mais consumida nas diferentes formas: processado ou *in natura*, no mundo. No Brasil, seu cultivo destaca-se por sua importância socioeconômica, principalmente, em função de seu valor econômico e por ser uma atividade geradora de empregos, melhorando a renda dos trabalhadores rurais e produtores (SOUZA et al., 2010).

Conforme os dados do último censo agropecuário do Instituto Brasileiro de Geografia e Estatística (IBGE, 2017), a produção nacional de tomate foi de 3.737.925 t, sendo a região Sudeste a maior produtora com 1.799.478 t de frutos (os maiores produtores são os estados de São Paulo e Minas Gerais) e em segundo e terceiro lugar despontam as regiões Centro-Oeste e Nordeste, respectivamente. Quanto à produtividade média, a região Nordeste ainda possui baixos índices (41,76 $t.ha^{-1}$) quando comparada à nacional (63,84 $t.ha^{-1}$), enquanto a região Sudeste apresenta valores superiores (67,55 $t.ha^{-1}$). Nessas duas regiões produtoras pode-se elevar os rendimentos, influenciados pela grande instabilidade climática e ocorrência de longos períodos de estiagem, o que dificulta o manejo adequado da cultura, afetando substancialmente o crescimento e o desenvolvimento das plantas.

Para se obter bons rendimentos e, consequentemente, retorno econômico com essa olerícola, é necessário manejo adequado da nutrição, fornecimento de água, cultivares melhoradas e sanidade (SILVA et al., 2013). Dentre esses fatores a água é um dos principais, pois, exerce maior influência nas características de crescimento, desenvolvimento e produção do tomateiro (SANTANA et al., 2009). A eficiência do uso da água é a relação entre a quantidade de produção agrícola obtida por área e o volume de água utilizada durante o cultivo para obtê-la. Para alcançar o seu máximo deve-se, então, reduzir a quantidade de água aplicada sem diminuir significativamente a produtividade ou aumentar a produção por área, utilizando a mesma quantidade de água de irrigação (CAMPAGNOL et al., 2018).

De acordo com Santana et al. (2010) o tomateiro é uma cultura exigente em tratos culturais, dentre os quais a irrigação exerce forte influência na produção e qualidade dos frutos, uma vez que é considerada sensível ao défice hídrico. Essa hortaliça é de grande importância para a economia agrícola nacional e a redução na utilização de água durante a fase vegetativa do seu cultivo permite economia de água e com a aplicação de AS aliada à essa redução na aplicação de água é possível manter os rendimentos satisfatórios das culturas, melhorando, assim, a eficiência do uso da água.

Estudos recentes demonstraram que a aplicação foliar de AS é capaz de mitigar os efeitos deletérios do défice hídrico em tomate do tipo grape, por meio do aumento do índice SPAD e teor de água na folha (CHAKMA et al., 2021). Hayat et al., (2008, 2010) mostram que a aplicação de AS melhorou a resposta de crescimento do tomateiro cultivado sob estresse hídrico. Senaratna et al. (2000) observaram que o AS apresentou resultados positivos em tomate cultivados sob estresse hídrico. O estudo de Ayub et al. (2022) reporta que a aplicação de AS está envolvida na estabilização e integridade celular de plantas de tomateiro sob ambiente de estresse térmico. O AS também está ligado à redução dos efeitos da toxicidade de metais pesados no solo, a aplicação deste regulador reduziu a peroxidação lipídica e aumentou a atividade das enzimas antioxidantes em plantas de tomateiro cultivadas em solo contaminado por cádmium (Cd) (GUO et al., 2018). Resultados apresentados por Silva et al. (2022) mostraram que a aplicação foliar de AS aumentou o crescimento, a produção, e a eficiência do uso da água de plantas de tomate cereja sob estresse salino induzido.

Assim, a hipótese dessa pesquisa é, portanto, que o ácido salicílico regula respostas bioquímicas, fisiológicas e produtivas em favor da mitigação de estresses em tomateiro em campo. E, para testar essa hipótese avaliamos as características agronômicas, fotossintéticas e bioquímicas de tomateiro do grupo italiano sob estreses com aplicações de ácido salicílico (AS).

Os dados coletados estão apresentados em forma de capítulos (artigos científicos já publicados), o primeiro “Foliar application of salicylic acid intensifies antioxidant system and photosynthetic efficiency in tomato” foi publicado na revista Bragantia https://doi.org/10.1590/1678-4499.20210320, e o segundo “Foliar application of salicylic acid to mitigate water stress in tomato” publicado na revista *Plants* https://doi.org/10.3390/plants11131775.

CAPÍTULO 1 - FOLIAR APPLICATION OF SALICYLIC ACID INTENSIFIES ANTIOXIDANT SYSTEM AND PHOTOSYNTHETIC EFFICIENCY IN TOMATO[1]

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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 intensifies antioxidant enzymes activities, chlorophyll *a* fluorescence, gas exchange and tomato production against environmental stress. This experiment was conducted by using the Colossal 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; 0.5; 1.0; 1.5 and 2.0 mM. SA and then, applications were performed weekly from 15 to 60 days after transplantation (DAT). At 45 and 60 DAT, the enzymes activities were analysed, such as SOD, CAT and 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 intensifies 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. thus, 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, increased photosynthetic efficiency and fruit production.

Keywords: photosynthesis, production, plant growth regulator, *Solanum lycopersicum*

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[1] Capítulo publicado na revista *Bragantia* https://doi.org/10.1590/1678-4499.20210320

1.1 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^{-1} by considering the average between field cultivation and protected environment (Faostat, 2020). 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 for the production of 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 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 de lipid peroxidation in *Brassica rapa* under drought stress (Lee et al. 2019).SA pre-treatment could considerably alleviate the harmful impact of heat and high light stress on PSII and helps to stimulate the rebuilding of photosynthetic function (Zhao et al. 2011). There are many research findings are available either individual effects of drought, salinity, high temperatures or salicylic acid (Chakma et al. 2021; Souri and Tohidloo, 2019; Jahan et al. 2019). 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 hypothesizes 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 salicylic acid application.

1.2 MATERIAL AND METHODS

The experiment was conducted in São Manuel (located in the homonymous town) Experimental Farm of Education, Research and Production from July to December 2019, this farm belongs to São Paulo State University (UNESP), School of Agriculture, Botucatu. The geographical coordinates are 22°44’S latitude, 47°34’W longitude and 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 1.0 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 27.0° C and 45% during the experimental period, respectively.

A randomized block design with five treatments and four blocks was used, each plot consisted of 4 useful plants. The treatments were 0 (control); 0.5; 1.0; 1.5 and 2.0 mM of salicylic acid, which were applied via foliar spray. In the treatments, the solutions were prepared from 5 mL of salicylic acid dissolved in absolute ethanol and supplemented with distilled water and, then, applied weekly for 7 weeks; that is, after 15 days of transplantation (DAT) until 60 DAT. The applications were made through a pressurized CO_2 manual sprayer (0.3 kgf per 31 cm^2) 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 ($CaCl_2$)= 4.2; M.O.= 9.0 g dm^{-3}; P(resin)= 2.0 mg dm^{-3}; H+Al= 31 $mmol_c$ dm^{-3}; K^+= 0.6 $mmol_c$ dm^{-3}; Ca^{2+}= 3.0 $mmol_c$ dm^{-3}; Mg^{2+}= 1.0 $mmol_c$ dm^{-3}; CTC= 36 $mmol_c$ dm^{-3} 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 45 and 60 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 (F0) was determined under sufficiently low irradiance (< 1 μmol m^{-2} s^{-1}). The maximal fluorescence (Fm) was determined after a 0.8-s saturation pulse at 4 200 μmol m^{-2} 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 (Fm′) was 6000 μmol m^{-2} s^{-1} for 0.8 s, whereas the actinic light was 200 μmol m^{-2} s^{-1}. Measurements of the quantum yield of photosystem (PS) II photochemistry (PSII) were obtained by application of a saturation light pulse (6000 μmol m^{-2} s^{-1} for 0.8 s) under ambient irradiance. The fluorescence parameters, such as variable maximum quantum yield of PSII (*Fv*/*Fm*), effective quantum yield of photosystem II (PSII) (*Fv*'/*Fm*'), 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 9:00 am and 11:00 am (Ramos et al. 2015). The CO_2 assimilation rate (*A*, μmolCO_2 m^{-2} s^{-1}), transpiration rate (*E*, mmol water vapour m^{-2} s^{-1}), stomatal conductance (g_s, mol m^{-2} s^{-1}) and internal CO_2 concentration in tomato leaf (*Ci*, μmolCO_2 mol^{-1} air), were measured in three plants per treatment with a portable open gas exchange system (LI-6400, LICOR). The CO_2 concentration entering the leaf cuvette (LCF chamber; 2 cm^2, LI-COR) averaged 400 μmol mol^{-1}, as provided by the 6400-01 CO_2 mixer (LI-COR). The photosynthetic photon flux density (PPFD) was provided by an artificial LED light source (6400-40 LCF, LI-COR; 90% red and 10% blue spectra), which was set to provide 1000 μmol photons m^{-2} 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 ± 0.18 kPa, which means the relative humidity in the (sample) chamber was 65.1 ± 2.3%; water use efficiency (*WUE*, μmolCO_2 [mmol H_2O^{-1}]) determined through the relations between CO_2 assimilation and transpiration rate; and the instant carboxylation efficiency (*A/Ci*) determined through the relation between CO_2 assimilation rate and the internal 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°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), 1% (m/v) polyvinylpolypyrrolidone, the homogenate was centrifuged at 15 000 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™ 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 U mg protein^{-1}.

To determine catalase activity (CAT), we followed the methodology proposed by Havir 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™ Microplate Elisa Spectrophotometer). One unit of CAT activity was defined as the degradation of 1μM of H_2O_2 for a minute. For CAT, the molar extinction coefficient (ε) was calculated as 36 mM^{-1} cm^{-1} in mKat mg protein^{-1}.

The activity of enzyme peroxidase enzyme (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™ Microplate Elisa Spectrophotometer). To calculate POD, it was used the molar extinction coefficient (2.5 mmol L^{-1} cm^{-1}) and activity expressed in μmol min^{-1} mg 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 was boiled at 95 °C for 30 min. The reaction mixture was cooled on ice after centrifugated at 10000 g for 5 min, the absorbance was measured at 532 and 600 nm wavelength, and the results expressed in μmol of MDA per gram of fresh weight.

The proline content was determined according to the method described by Bates, Waldren and Teare (1973), sample of macerated fresh leaves (100 mg) was homogenized in 1.5 mL of sulphosalicylic acid 3% and after centrifugated at 10000 g for 5 min. For colorimetric determinations, a solution of extract, ninhydrin acid and glacial acetic acid was incubated at 90 °C for 1h. 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 μmol of L-proline per gram of fresh weight.

For measuring tape was used for plant height, the harvest started at 75 DAT and ended at 120 DAT, the fruits of each plot were separated into commercial and non-commercial according to the Tomatoes Standard Classification (Cqh/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 commercial production of fruits kg 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® program. Sigmaplot® program helped to create the graphics.

1.3 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, 45 and 60 DAT. As for the chlorophyll *a* fluorescence parameters analyzed, there was a significant effect ($p<0.05$) at 45 DAT for: *qNP*, *ETR* and *Fv*/*Fm*; and at 60 DAT, only, *ETR* showed this effect. For gas exchange, all variables were affected ($p<0.05$) by the application of SA at 45 DAT: *A*, g_s, *Ci*, *E*, *WUE* and *A*/*Ci*; at 60 DAT there was a significant effect ($p<0.05$) for: *A*, g_s, *Ci* and *A*/*Ci*. Height evaluations at 45 and 60 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 (Figure 1a and b), while the activity of POD showed a quadratic effect (Figure 1c). Such behaviour reduced lipid peroxidation by approximately 70% from 0.5 mM SA at 45 DAT. For the proline content, the application of 0.90 mM SA caused a greater accumulation, that is, 785 µmol g^{-1} FW at 45 DAT (Figure 1e).

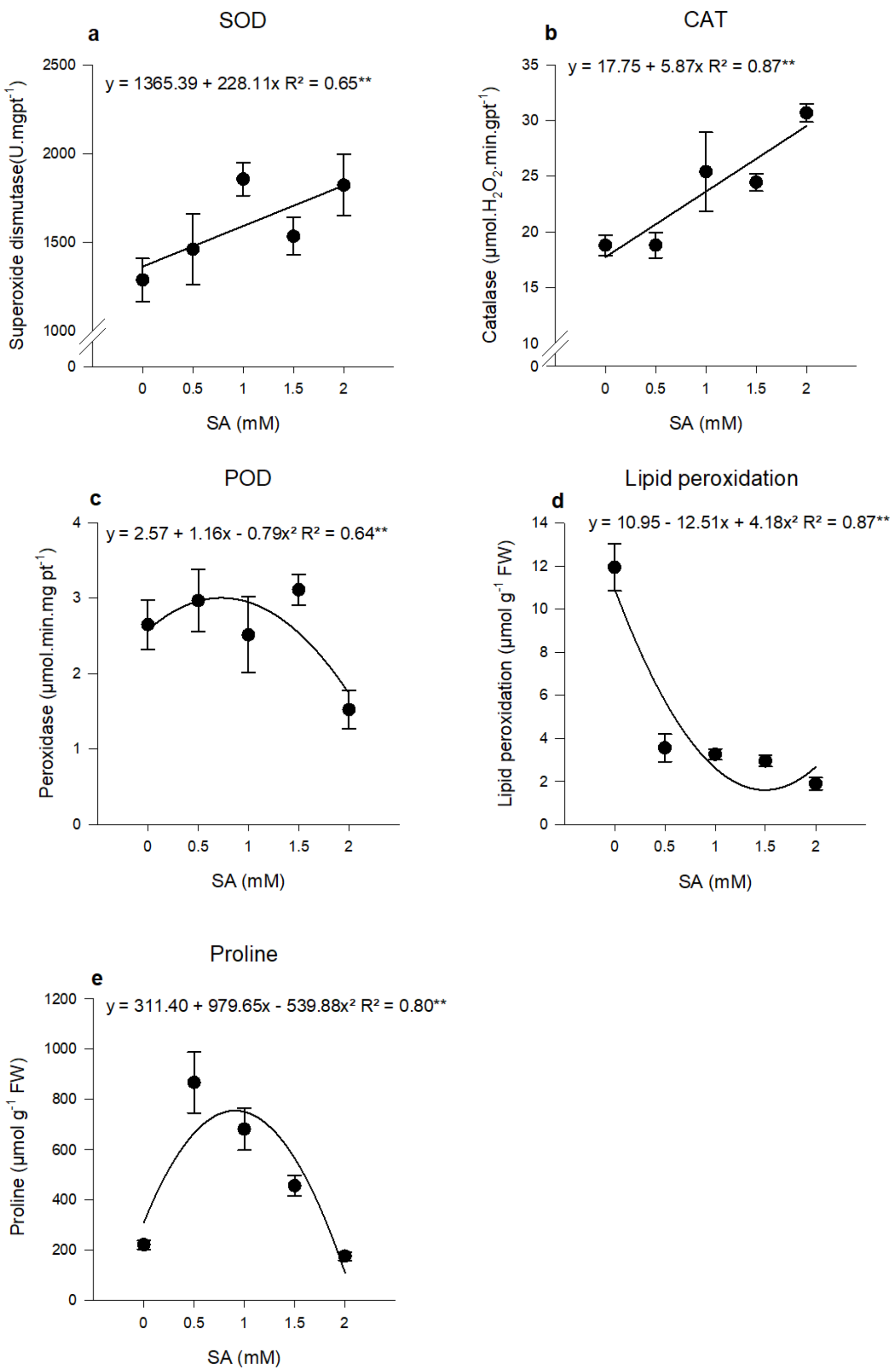

Figure 1. Activity of enzymes superoxide dismutase - SOD (a), catalase - CAT (b) and peroxidase - POD (c); lipid peroxidation (d) and proline content (e) in tomato plants subjected to salicylic acid (SA) application at 45 DAT. Botucatu, SP, 2020. Whiskers represent 95% confidence intervals of means. ** $0.01 < p < 0.05$.

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 showed significant differences and quadratic equations in the *qNP*, *ETR* and *Fv/Fm* at 45 DAT. The *qNP* showed lower value at 1.25mM SA, that is, a 25% reduction in the dissipated energy in a non-photochemical way compared to control (Figure 2a). The application of 1.0 mM SA caused higher values for *ETR* and *Fv/Fm* (Figure 2b and c). Moreover, the SA application positively influenced all gas exchange variables at 45 DAT, such as *A*, g_s, *E*, *Ci* and *A*/*Ci* (Figure 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 µmol m^{-2} s^{-1} (Figure 3a), the same dose also increased the rate of stomatal opening to 0.27 mol m^{-2} s^{-1} (Figure 3b) and transpiration rate to 6.54 mmol m^{-2} s^{-1} (Figure 3d). At 45 DAT, the *Ci* values (Figure 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 (Figure 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 at 60 DAT (Figure 4 a, b e c) with the dose of 2.0mM SA and, consequently, lipid peroxidation decreased in plants exposed to this treatment (Figure 4d). The proline content in tomato leaves followed the trend of enzyme activity values, that is, the dose of 2.0mM enabled greater accumulation in plants (Figure 4e).

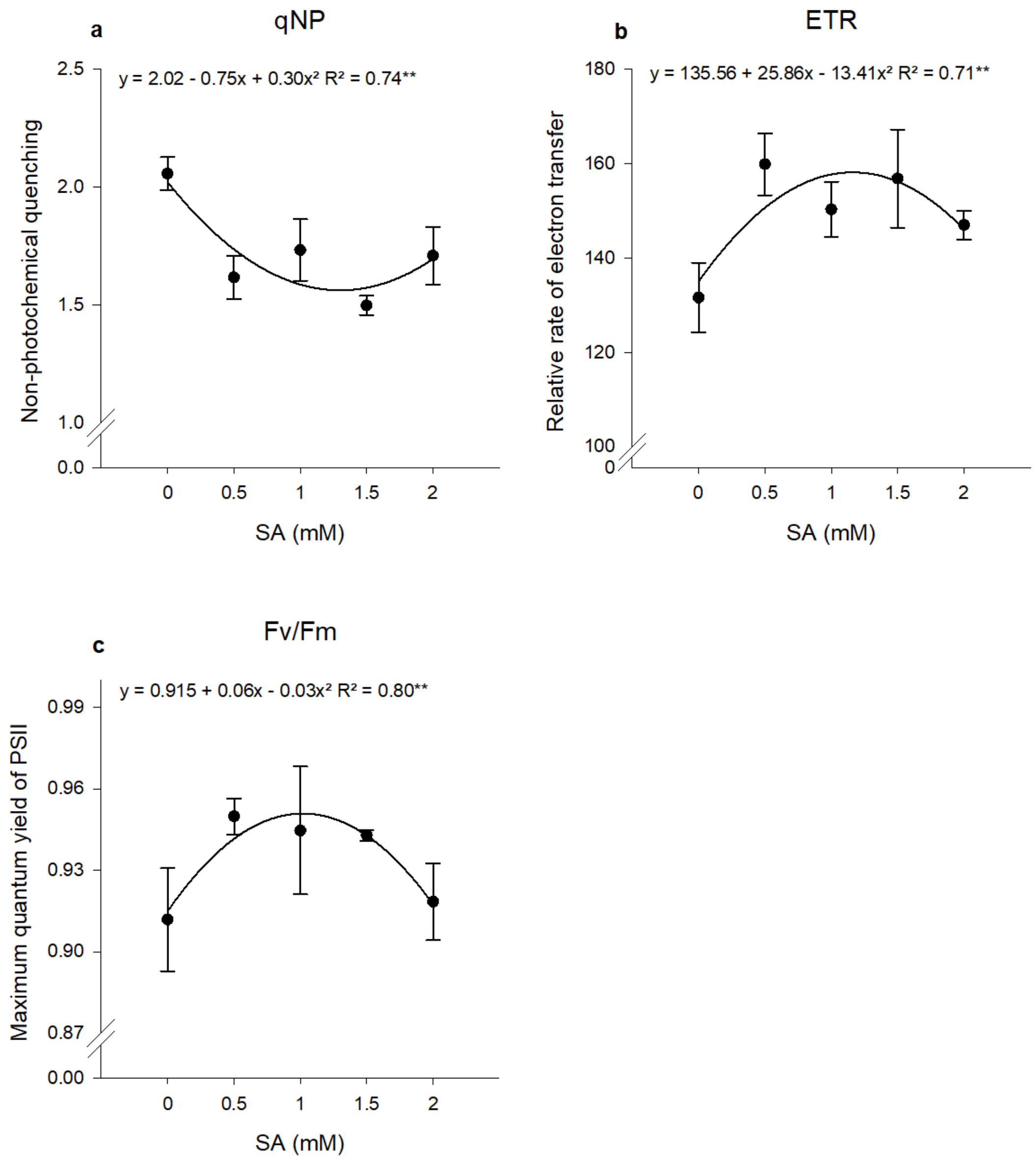

Figure 2. Non-photochemical quenching - *qNP* (a), relative rate of electron transfer - *ETR* (b) and maximum quantum yield of PSII - *Fv/Fm* (c) in tomato plants subjected to salicylic acid (SA) application at 45 DAT. Botucatu, SP, 2020. Whiskers represent 95% confidence intervals of means. ** $0.01 < p < 0.05$.

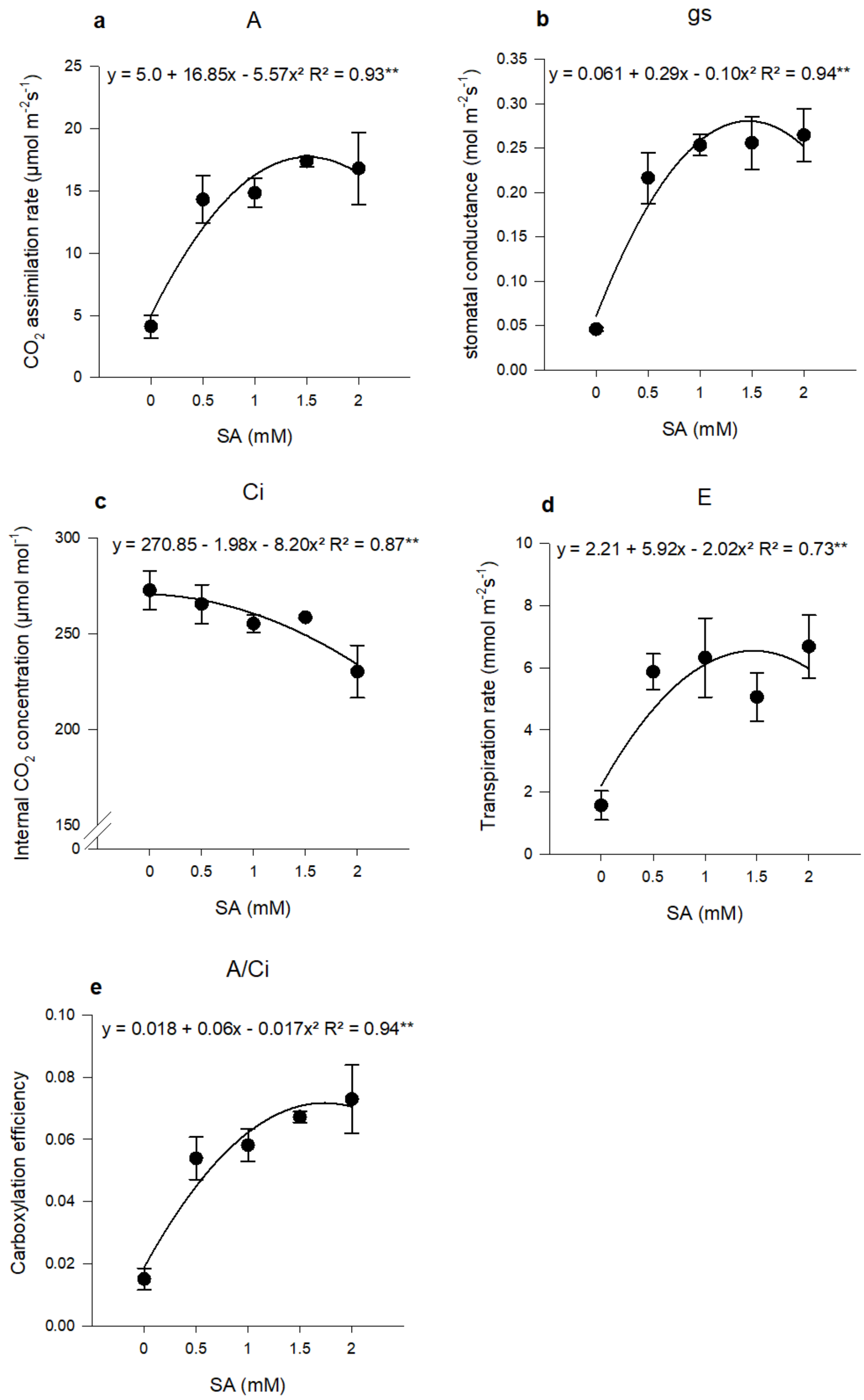

Figure 3. CO_2 assimilation rate - *A* (a), stomatal conductance - g_s (b), internal CO_2 concentration in the leaf - *Ci* (c), transpiration rate - *E* (d) and carboxylation efficiency - *A/Ci* (e) in tomato plants subjected to salicylic acid (SA) application at 45 DAT. Botucatu, SP, 2020. Whiskers represent 95% confidence intervals of means. ** $0.01 < p < 0.05$.

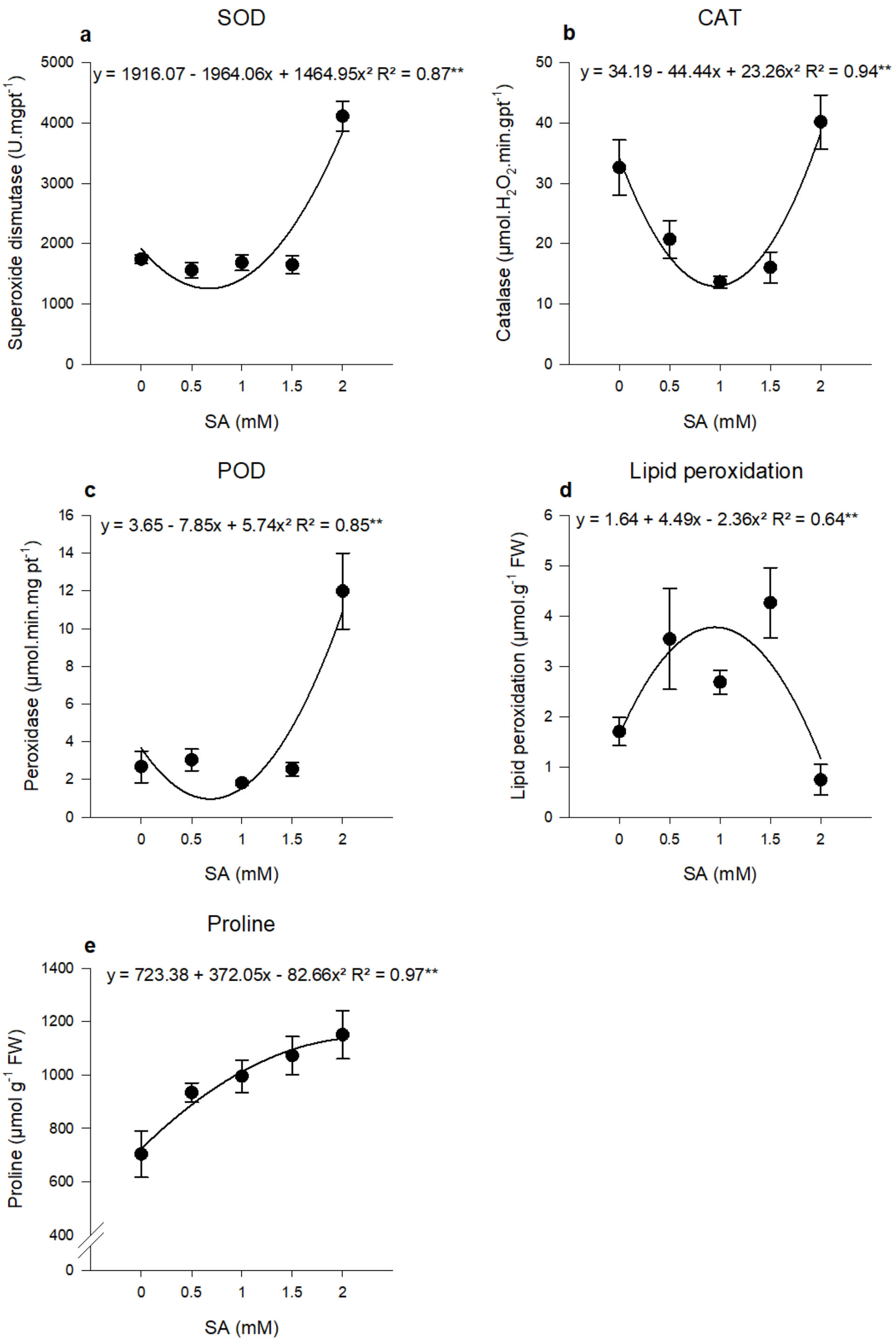

Figure 4. Activity of enzymes superoxide dismutase - SOD (a), catalase -CAT (b) and peroxidase - POD (c); lipid peroxidation (d) and proline content (e) in tomato plants subjected to salicylic acid (SA) application at 60 DAT. Botucatu, SP, 2020. Whiskers represent 95% confidence intervals of means. ** 0.01 < p < 0.05.

With regards to the analysis of chlorophyll *a* fluorescence, *ETR* was the only one influenced by SA application at 60 DAT (Figure 5a). The higher *ETR* values directly reflected on higher CO_2 assimilation rate in tomato plants treated with 1.01- and 1.4-mM SA, respectively (Figure 5b). Most gas exchange variables were positively influenced by the action of the exogenous application of SA at 60 DAT, except transpiration rate. Similar behaviour happened in the evaluations at 45 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_s followed the same behaviour as *A* with a quadratic tendency; and 2.00 mM SA promoted greater stomatal opening to 0.1193 mol m^{-2} s^{-1} (Figure 5c), while *Ci* 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 (Figure 5d and e).

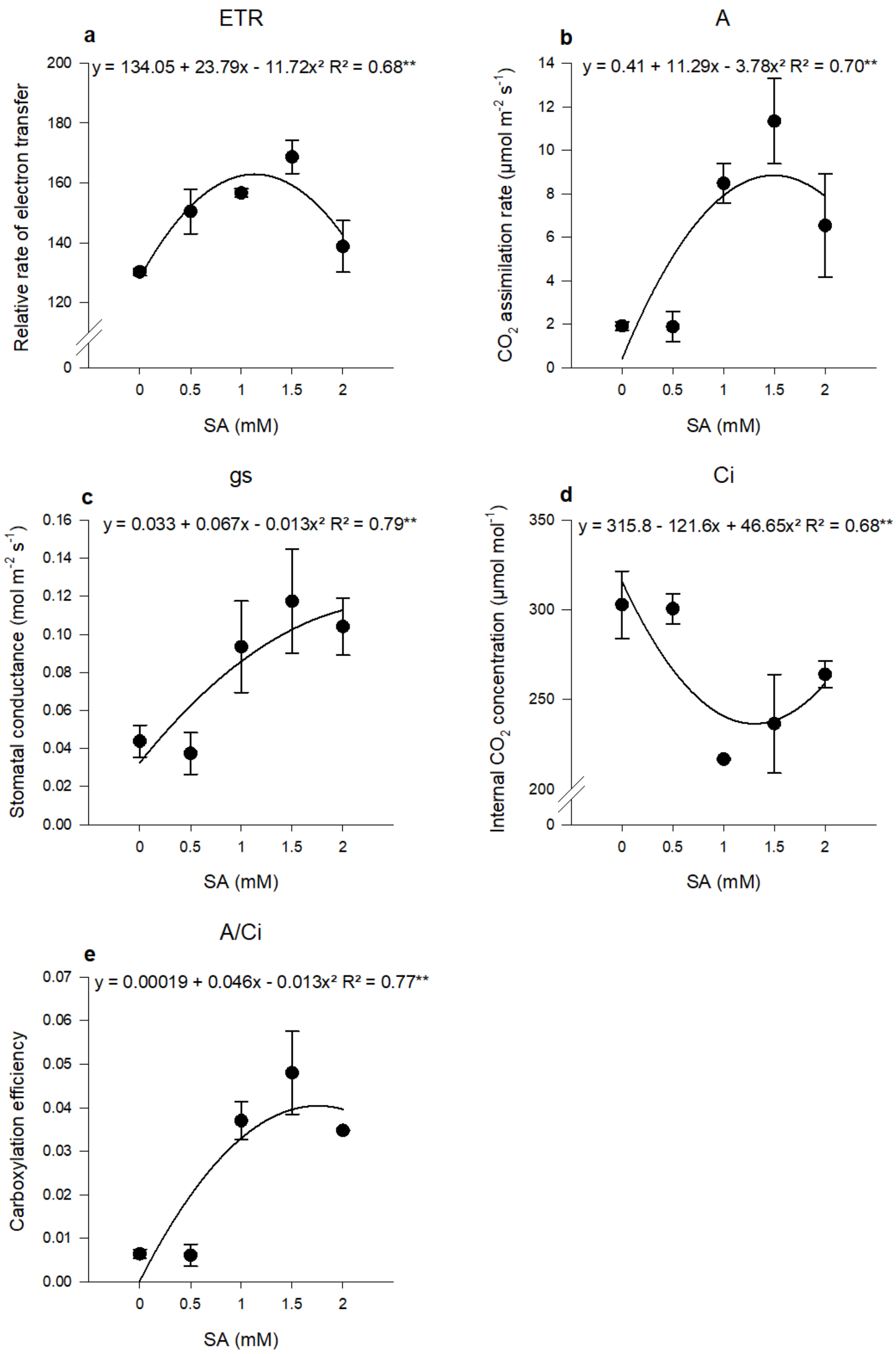

Figure 5. Relative rate of electron transfer - *ETR* (a), CO_2 assimilation rate - *A* (b), stomatal conductance - g_s (c), internal CO_2 concentration in the leaf - *Ci* (d), transpiration and carboxylation efficiency - *A/Ci* (e) in tomato plants subjected to salicylic acid (SA) application at 60 DAT. Botucatu, SP, 2020. Whiskers represent 95% confidence intervals of means. ** $0.01 < p < 0.05$

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 45 and 60 DAT (Figure 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 the experiment, it improved the final fruit production, because of the increase in the fruit weight to 136.0 g at 0.82 mM SA (Figure 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^{-1} at 0.80 mM SA and a commercial fruit production of 2.90 kg plant^{-1} at 0.5 mM SA (Figure 7b).

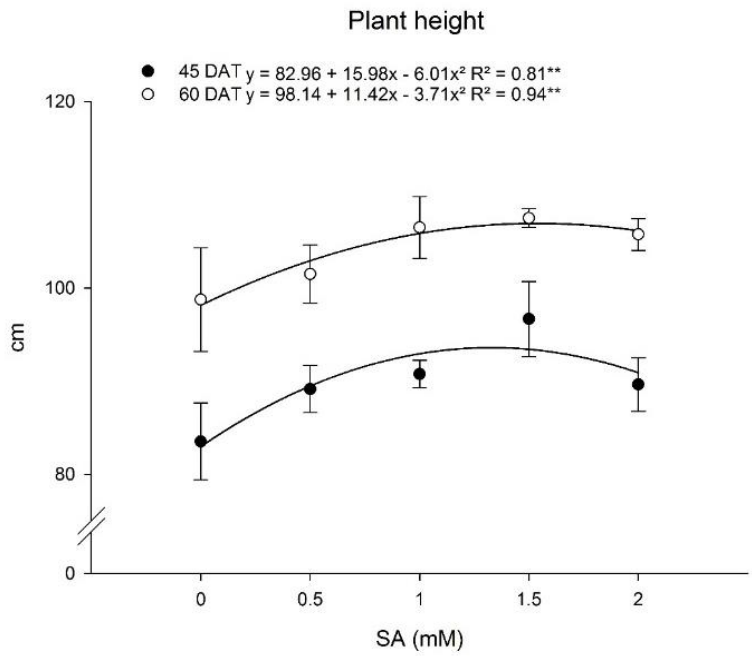

Figure 6. Plant height in tomatoes plants subjected to salicylic acid (SA) application at 45 and 60 DAT. Botucatu, SP, 2020. Whiskers represent 95% confidence intervals of means. ** 0.01 < p < 0.05.

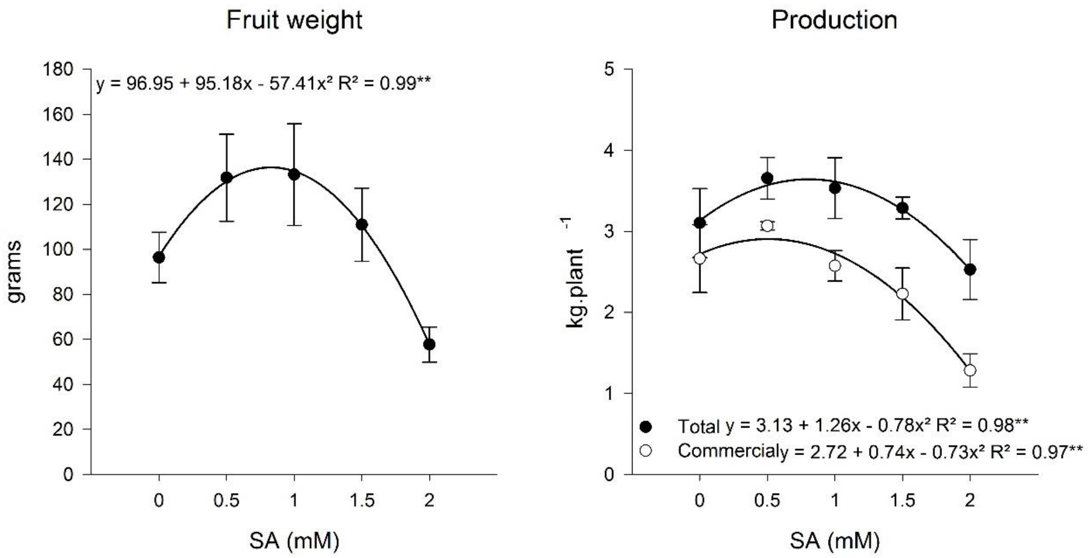

Figure 7. Fruit weight, total and commercial production of tomatoes fruits in plants subjected to salicylic acid (SA) application. Botucatu, SP, 2020. Whiskers represent 95% confidence intervals of means. ** $0.01 < p < 0.05$.

1.4 DISCUSSION

Our results suggest that foliar application of salicylic acid 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 osmorregulator, ensuring high g_s and A and increasing the photoassimilates. The tomato plants treated 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 (Martin-Mex et al. 2012) and petunia (Martin-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.

Note that the mechanism of action of SA is not well understood, mainly because it can differ from species to species, as well as vary 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_2 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 (1.0 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 – 2.0 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, 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 45 and 60 DAT. Nevertheless, oxygen-dependent metabolic processes, such as aerobic respiration, photosynthesis, and photorespiration lead to the production of reactive oxygen species (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 osmorregulators, 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 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 $NADPH_2$, and these products, in turn, are consumed in the Calvin-Benson cycle, by reactions catalysed by enzymes that reduce atmospheric CO_2 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 & Weis, 1991). The *Fv*/*Fm* reduction can be an indicative of photo inhibitory damage caused by the incidence of photon flux density when plants are subjected to a wide range of environmental stresses (Björkman and Demming 1987). As the *Fv/Fm* values observed in tomato plants treated with SA (0.5 and 1.0 mM) were higher than 0.95, it is 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*, where 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 $NADPH_2$ and ATP. The role of exogenous SA on the characteristics of chlorophyll a fluorescence in tomato plants it is the maintenance of the integrity of the photosynthetic apparatus by raising *Fv/Fm* and *ETR*, in time, minimizing the excess 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_s promoted by the SA allowed greater availability of CO_2 in leaf mesophyll cells; thus, increasing *A*, *E* and *A*/*Ci*. However, *Ci* was reduced while g_s increased, this may be an indication that the increase in *A* occurs due to the higher g_s 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_2 during the photosynthetic process, related to the greater g_s that allowed a higher *A* value.

The relationship between the application of SA and g_s 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 et al. 1992; Sehar et al. 2020) and stimulates stomatal closure via second messengers such as reactive oxygen species (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_s in tomato plants treated with SA.

Even if treatment increases g_s and *A*, the plant depends on internal CO_2 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., where 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_2 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). These authors 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 photoassimilates were translocated to the fruit, that lead the greater weight and yield.

1.5 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/Ci*; consequently, reflecting on plant height and weight accumulation in fruits.

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CAPÍTULO 2 - FOLIAR APPLICATION OF SALICYLIC ACID TO MITIGATE WATER STRESS IN TOMATO[2]

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ABSTRACT: Salicylic acid (SA) is an important plant regulator reported as a mitigator of water deficit in plants, however without a recommendation for use in field conditions. Thus, this research aims to validate the use of SA under field conditions in regions with low water availability. For that, we evaluated CO_2 assimilation (*A*), stomatal conductance (g_s), transpiration (*E*), water use efficiency (*WUE*), and carboxylation efficiency (*A*/*Ci*) at 15, 30, and 45 days of continuous stress water deficit, as well as the application of salicylic acid (0.0; 0.5; 1.0; 1.5; 2.0 mM) in tomato plants subjected to continuous water deficit (45 days), in two years (2019 and 2020). The water deficit reduced the *A*, g_s, *E* and *A*/*Ci*, while the foliar application of SA increased these parameters in all evaluated times, resulting in similar or even higher values than in plants without water deficit. Water deficit caused floral abortion in tomato plants, without the application of SA, reducing the number of fruit production. In contrast, plants that received about 1.3 mM of SA increased *A* and *A*/*Ci* and translocated the photoassimilates, mainly to flowers and fruits, reducing floral abortion and increasing fruit production. Thus, foliar application of SA was efficient in mitigating the deleterious effects of water deficit in tomato plants regarding the gas exchange and fruit production.

KEYWORDS: floral abortion; photosynthesis; plant regulation; *Solanum lycopersicum*; water deficit

[2] Capítulo publicado na revista *Plants* https://doi.org/10.3390/plants11131775

2.1 INTRODUCTION

Climate Change has become a challenge for global food security [1], as rising temperatures and reduced water availability decrease crop productivity. Future scenarios indicate that water shortages will affect 50% of agricultural land by 2050 [2]. Besides, water deficit is the biggest environmental constraint that limits field crops and vegetables [3,4].

Among other culinary vegetables, the tomato crop is sensitive to water restriction, negatively affecting growth, production, and fruit quality [5]. Due to its high commercial demand, tomato cultivation is widespread worldwide, being cultivated on more than 5.03 million hectares, both in the field and under-protected cultivation [6].

Since tomato cultivation is mostly carried out in the open field, one of the factors that most affect its development is water availability. Thus, water deficit affects plant growth and development by negatively impacting cell division and elongation generating adverse effects on plant physiology, morphology, and ecology [7]. The limitation in growth occurs mainly because it causes a reduction in photosynthesis. This reduction results from the decrease in the activity of the enzyme ribulose 1,5-bisphosphate carboxylase/oxygenase (RuBisCo), caused by the limitation of the amount of CO_2 in the intercellular space [8,9], which occurs due to stomatal closure.

With low water availability, stomatal closure occurs as an early response [10]. Stomatal functionality is regulated by hydraulic signals [11], although, under water deficit conditions, stomata also respond to chemical or hormonal signals produced by dehydrated roots. The most important hormonal signal in this regard is abscisic acid (ABA), found in high concentrations in plants under water deficit conditions [12]. As hormonal signaling occurs for stomatal closure and cascading effect on CO2 assimilation by the plant, a possible alternative to mitigate the deleterious effects of water deficit in plants is the application of plant regulators. Advances in agronomical practices, traditional breeding and modern biotechnological tools have been used to prevent the yield losses due to drought stress [13]. Adapting crops to drought stress would be the most economical strategy to improve water use efficiency and crop productivity [14].

Exogenous application of phytohormones and biostimulants is known as the effective adapting methods [15,16]. Among this salicylic acid (SA) can be mentioned, which is a phenolic compound with action on plant growth, ion absorption, and substance transport. SA is considered an important plant signaling molecule for the defense response of plants, increasing plant tolerance to biotic and abiotic stresses [17,18].

SA is part of several plant processes and its role in mitigating biotic or abiotic stresses has already been reported. For example, water deficit reduced stomatal conductance, transpiration, and CO_2 assimilation in rice plants, while SA application increased gas exchange characteristics in plants under water deficit [19]. Habibi (2012) also demonstrated that net photosynthetic rate, transpiration rate, and stomatal conductance increased in barley plants under water deficit treated with SA [20].

Positive effects of SA application were observed on the growth and biomass accumulation of Portulaca oleracea under water deficit due to the maintenance of photosynthetic pigments and increased CO_2 assimilation [21]. Chavoushi et al. (2020) reported that treatment with SA in safflower (Carthamus tinctorius L.) under water deficit improved photosynthesis rate, anthocyanin content, and phenylalanine ammonium lyase (PAL) enzyme activity however, it did not affect the accumulation of plant dry matter [22]. Recent results have shown that the foliar application of SA in grape tomatoes acts as water deficit mitigation [5]. However, grape tomatoes are not the most cultivated worldwide and also, as experimental conditions of this study cannot be replicated at the field level, that raises the importance of validating SA doses in situations that farmers can apply.

Natural biostimulants as eco-friendly materials include any substances applied to plants to enhance nutritional efficiency, abiotic stress tolerance, and/or crop quality traits [23]. Hence, considering that the action mechanism of SA can differ between plant species, stress levels, environmental conditions and others, it is important to evaluate the effect of SA on tomato plants under water deficit in field conditions to make the production of this culinary vegetable more sustainable, as well as to suggest options for cultivation in the regions with low water availability.

Based on the research reports, our research hypothesizes that foliar application of salicylic acid can reduce the deleterious effects of water deficit on gas exchange and tomato fruit yield. To test this hypothesis, we evaluated CO_2 assimilation, stomatal conductance, transpiration, water use efficiency, carboxylation efficiency, and productivity of tomato plants subjected to water deficit under the application of different doses of salicylic acid.

2.2 MATERIALS AND METHODS

2.2.1 Plant Material and Cultivation Conditions

The experiment was carried out at the Teaching, Research and Production Farm of São Manuel, in São Manuel city, São Paulo state, belonging to the School of Agronomic Sciences, Botucatu Campus, Sao Paulo State University, UNESP (22◦440 S de, 47◦340 W).

The climate of the region is classified as humid subtropical mesothermal. Pots (15 dm3) were filled with a Red Yellow Latosol, in sand phase, collected on the farm, with the following characteristics: pH ($CaCl_2$) = 4.2; MO = 9.0 g dm^{-3} ; P(resin) = 2.0 mg dm^{-3}; H + Al = 31 mmolc dm^{-3} ; K+ = 0.6 mmol dm^{-3} ; Ca^{2+} = 3.0 $mmol_c$ dm^{-3} ; Mg^{2+} = 1.0 $mmol_c$ dm^{-3} ; CTC = 36 $mmol_c$ dm^{-3} , and V = 12%.

Soil treatment was performed with dolomitic limestone, foundation fertilization with simple superphosphate, potassium chloride, and urea. Fertigation was carried out with calcium nitrate, potassium chloride, magnesium sulfate, and monoammonium phosphate (MAP), as described [24]. The temperature and relative humidity of the air was monitored inside the greenhouse during the experimental period (Figure 1)

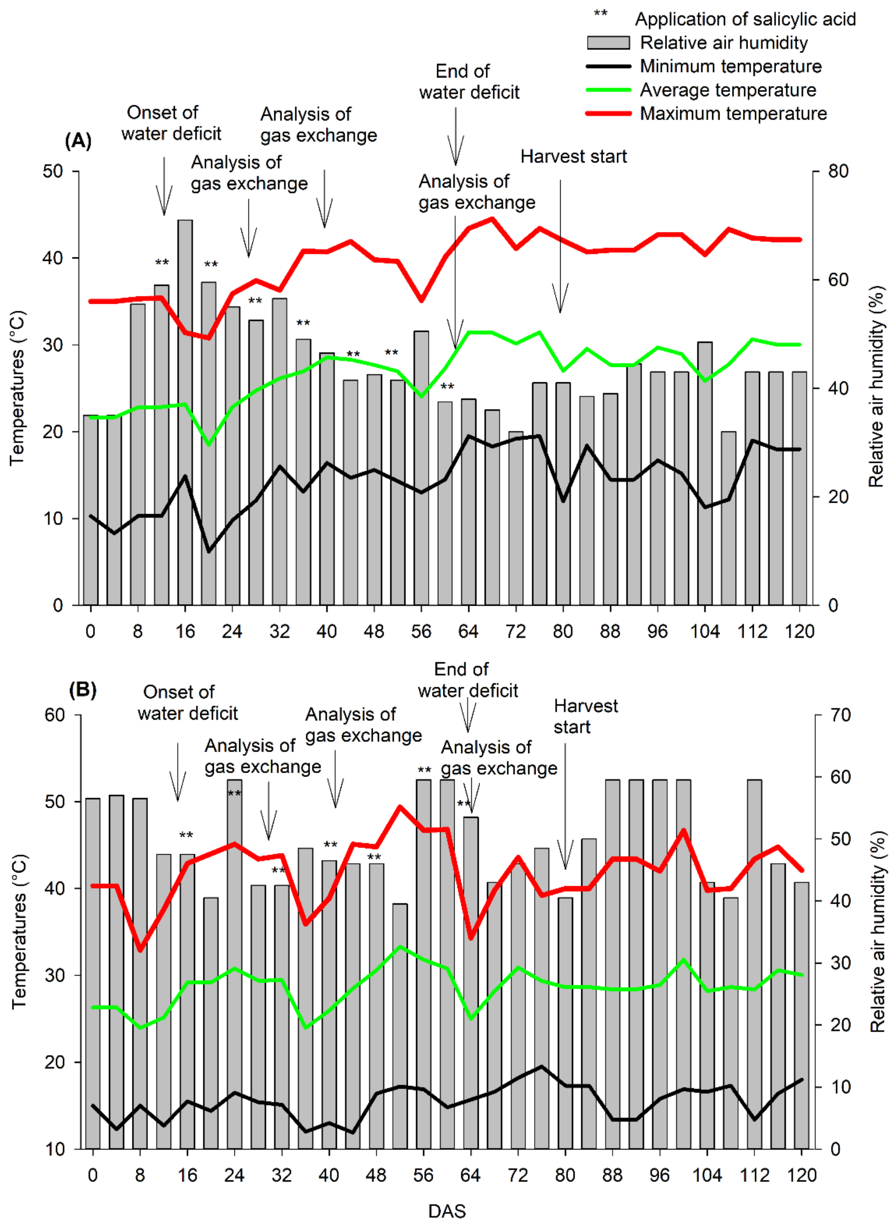

Figure 1. Average, maximum, and minimum temperatures (°C); Relative air humidity (%) inside the greenhouse during 2019 (**A**) and 2020 (**B**) cycles. DAS—Days after sowing.

2.2.2 Treatments

Two production cycles were conducted between July and December 2019 and 2020. The experiment was carried out in randomized blocks with six treatments: application of five doses of SA (0.0; 0.5; 1.0; 1.5; 2.0 mM) in plants under water deficit with 70% of ETc (n = 4) and the control with 100% crop evapotranspiration (ETc) without application of salicylic acid (SA), four blocks were used, each plot consisted of four useful plants. For irrigation management and imposition of treatments under water deficit, the water depth was defined by measuring the vessel capacity by weighing lysimetry, with irrigation applied daily to maintain this capacity, based on the plants submitted to full irrigation (100% of ETc). Water deficit started at 15 days after sowing (DAS) and ended at 60 DAS, totaling 45 days of exposure to water restriction. The period was defined in a first test trial, where plants under continuous water deficit did not withstand a period longer than 45 days of water limitation. After 60 DAS, plants were irrigated at total capacity until the harvest (120 DAS).

The seedlings were transplanted into 15 dm3 pots when they were on average 0.15 m high, with a spacing of 1.0 m between rows and 0.5 m between each plant in a row, conducted vertically and tutored with the help of bamboos. The experiments used the tomato hybrid Colossal, of the Italian type of determined growth, developed by the company Sakata Seed Sudamérica®. The Italian type of tomato is one of the most consumed consumers in the world, and it is cultivated in open fields or a protected environment, we chose this variety because it is representative of cultivation in different regions and because it has resistance to: Tomato Yellow leaf curl begomovirus (TYLCV), tomato spotted wilt tospovirus (TSWV), *Meloidogyne incognita* (Mi), *Meloidogyne javanica* (Mj), *Fusarium oxysporum* f. sp. *lycopersici* 1, 2 e 3. It is also not drought tolerant.

2.2.3 Foliar Application

Solutions containing salicylic acid for each treatment were prepared by dissolving salicylic acid in 5 mL of absolute ethanol, topped up with distilled water and applied from 15 DAS weekly, until 60 DAS, totaling 7 applications. Applications were carried out using a manual CO_2 pressurized sprayer, with 0.3 kgf per 31 cm^2 with full conical nozzles. In each application, 35 mL of the treatment solution was applied per plant. The plants under water deficit and without application of SA and the control plants received an application of water and 5 mL of absolute ethanol in the same volume as applied to the plants treated with SA.

2.2.4 Gas Exchange

The photosynthetic parameters related to gas exchange were evaluated at 15, 30, and 45 days of water deficit (DWD). For this purpose, fully expanded leaves were selected in the middle third of the plant and then readings were taken between 8:00 and 11:00 am.

The CO_2 assimilation rate (*A*, µmol CO_2 $m^{-2}.s^{-1}$), transpiration rate (*E*, mmol water vapor $\cdot m^{-2}.s^{-1}$), stomatal conductance (g_s, mol$\cdot m^{-2}.s^{-1}$), and internal CO_2 concentration in tomato leaf (*Ci*, µmolCO_2 $\cdot mol^{-1}\cdot$air) were measured in three plants per treatment with a portable open gas exchange system (LI-6400, LICOR). The CO_2 concentration entering the leaf cuvette (LCF chamber; 2 cm^2, LI-COR) averaged 400 µmol.mol^{-1}, as provided by the 6400-01 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 1000 µmol photons$\cdot m^{-2}$ $\cdot s^{-1}$in the leaf cuvette, based on the curve of light performed previously. The vapor pressure deficit (VPD) inside the leaf cuvette was 2.08 ± 0.18 kPa, which means the relative humidity in the (sample) chamber was 65.1 ± 2.3%; water use efficiency (*WUE*, µmolCO_2 [mmol H_2O^{-1}]) determined through the relations between CO_2 assimilation and transpiration rate; and the instant carboxylation efficiency (*A*/*Ci*) determined through the relation between CO_2 assimilation rate and the internal CO_2 concentration of in tomato leaf.

2.2.5 Productive Characteristics

The fruit harvest started at 75 DAS and ended at 120 DAS, with the fruits of each pot being separated into commercial and non-commercial according to the Tomato Classification Norms [25] and then counted, thus determining the total number (TNF) and commercial number (CNF) of fruits. Afterwards, the fruits were weighed with a scale, determining the mass of the fruits and, by the sum of the production of each harvest, the total and commercial production of fruits in kg $plant^{-1}$ was determined.

2.2.6 Statistical Analysis

The data obtained in the 2019 and 2020 cycles were analyzed separately, without considering them as a factor and the dates of gas exchange analysis.

The data were previously submitted to the Anderson Darling homogeneity test for statistical analysis using the Minitab software. Once the normality of the data was verified, analysis of variance (F Test) and regression analysis was performed, using the R statistical software. The control treatment (no application of SA and no water deficit) was compared to the other treatments through Dunnett's Test, adopting a significance level of 5% probability ($p < 0.05$) on Sigma Plot 11 software [26].

2.3 RESULTS

The results indicated a significant effect of SA on tomato plants under water deficit for all variables evaluated at different times. In a preliminary test, it was observed that the maximum period of water deficit (70% ETc), under the conditions of this research, was 45 continuous days and, for this reason, the restriction imposition was 45 days at the beginning of tomato development (critical period for establishment and production of the crop). Starting from this period, we evaluated the effect of SA on gas exchange in plants at 15, 30 and 45 days of water deficit (DWD) to understand the action of SA during the stress caused by water deficit and the dose needed to mitigate the deleterious effects of this stress on tomato photosynthesis.

2.3.1 Gas Exchange

Foliar application of salicylic acid positively and significantly affected all evaluated gas exchange characteristics: CO_2 assimilation (*A*), stomatal conductance (g_s), transpiration (*E*), the internal concentration of CO_2 (*Ci*), water use efficiency (*WUE*), and carboxylation efficiency (*A*/*Ci*), in the regular evaluations at 15, 30, and 45 DWD, in the two consecutive years (2019 and 2020).

For *A* (Figure 2 A,C,E), increments were observed up to the maximum point curve, 1.4 and 1.2 mM of SA at 15 DWD, in the two cycles, respectively. On that evaluation date, the use of SA at the mentioned doses, under water deficit, provided similar CO_2 assimilation for plants without water restriction (Figure 2A). At 30 DWD

(Figure 2C), the dose of 1.4 mM of SA was the one that provided the highest *A* for the two years of cultivation when compared to plants without water deficit. Plants that received this dose of SA showed higher CO_2 assimilation. For the last evaluation, at 45 DWD (Figure 2E), the dose of 0.9 mM of SA favored higher *A* to the point that plants under water deficit showed similar values to plants without water deficit in 2019. In 2020, the dose of 0.5 SA mM gave higher results for *A*, reaching values higher than those of plants without water restriction.

The g_s, also increased with the 1.4 mM and 1.1 mM doses of SA at 15 DWD (Figure 2B). In 2019, the application of SA at all tested doses caused stomatal conductance like that of plants without water restriction. In 2020, g_s was higher in plants treated with SA when compared to plants without water restriction.

At 30 DWD (Figure 2D), following the response and inflection point adjustment, the doses of 2.0 mM and 1.1 mM of SA caused greater stomatal opening in plants under water deficit in 2019 and 2020 respectively. In 2019, plants that received a dose greater than 0.5 mM of SA showed higher g_s when compared to plants without water restriction. In 2020, the application of SA generated greater g_s in plants under water deficit when compared to those of the control group.

The doses of 1.1 and 0.6 mM of SA provided higher g_s at 45 DWD (Figure 2E) in tomato plants under water deficit in 2019 and 2020, respectively. Due to the stomatal opening, CO_2 enters the substomatic chamber. Thus, the carbon of the CO_2 will be assimilated, and this fact can be verified by the variable *Ci* (Figure 3 A,C,E), in which lower values were expected to indicate the best treatments.

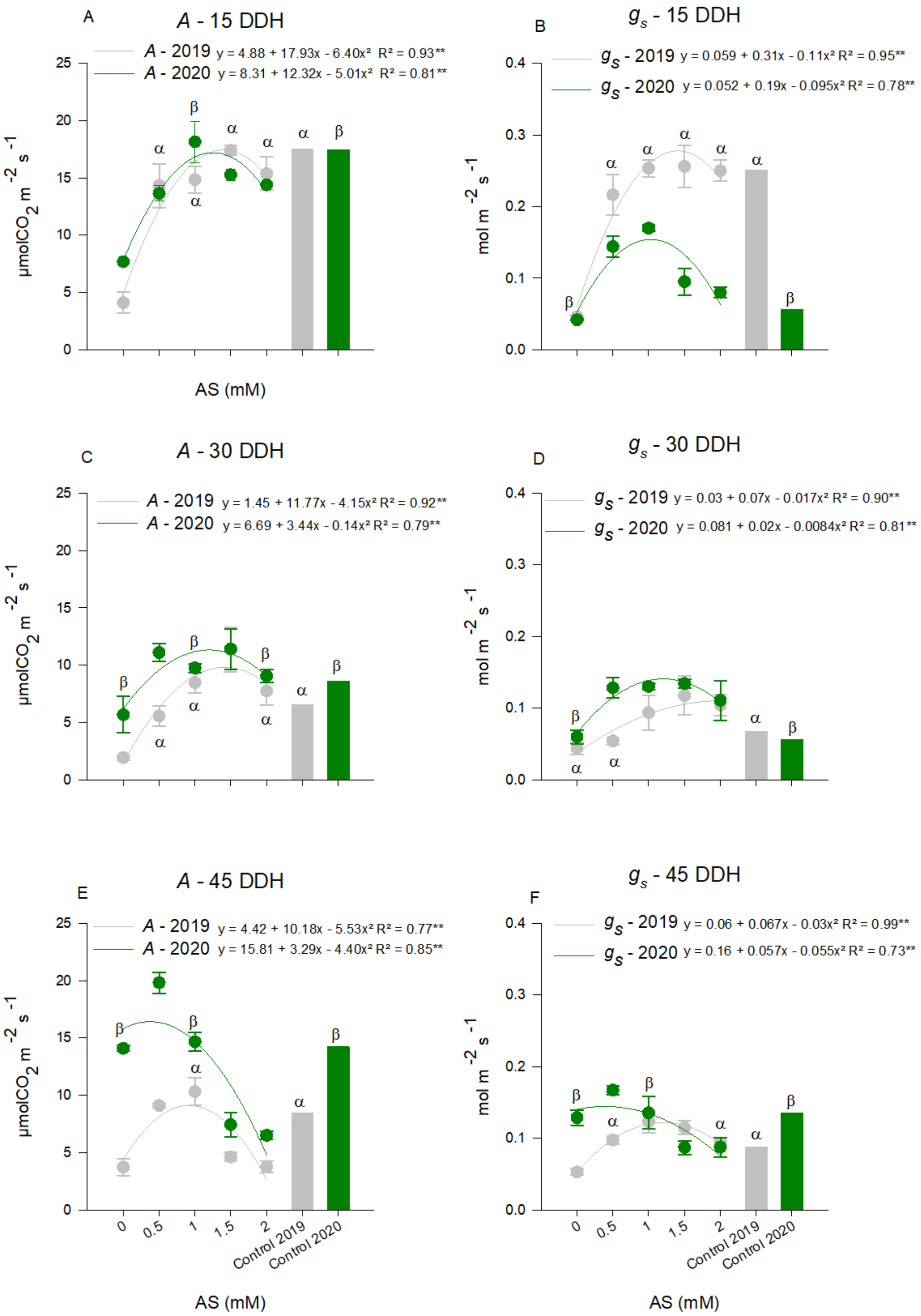

Figure 2. CO_2 assimilation rate (*A*) at 15 (**A**), 30 (**C**), and 45 (**E**) days of water deficit (DWD); stomatal conductance (g_s) at 15 (**B**), 30 (**D**), and 45 (**F**) DWD in tomato plants subjected to application of salicylic acid (SA) doses in two consecutive years, 2019 and 2020. ** Highly Significant at 5% probability. $^{\alpha}$ Indicates an equal and significant effect between treatments by Dunnett's Test at 5% probability in the 2019 cycle and $^{\beta}$ in the 2020 cycle. The bars show the standard deviation. *n* = 4 (number of replicates).

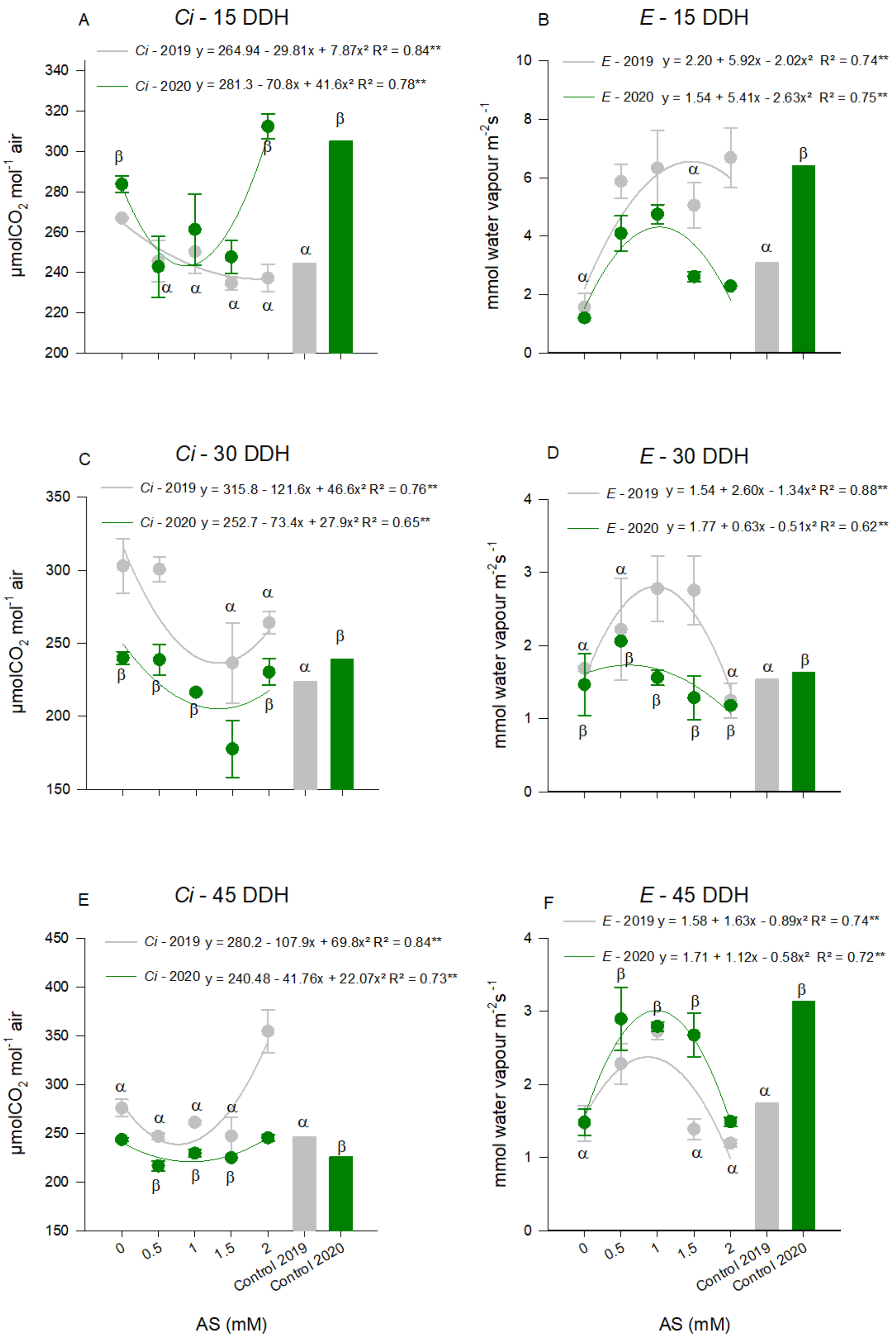

Figure 3. Internal concentration of CO_2 (*Ci*) at 15 (**A**), 30 (**C**), and 45 (**E**) days of water deficit (DWD); transpiration (*E*) at 15 (**B**), 30 (**D**), and 45 (**F**) DWD of tomato plants subjected to application of salicylic acid (SA) doses in two consecutive years 2019 and 2020. ** Highly Significant at 5% probability. $^{\alpha}$ Indicates an equal and significant effect between treatments by Dunnett's Test at 5% probability in the 2019 cycle and $^{\beta}$ in the 2020 cycle. The bars show the standard deviation. *n* = 4 (number of replicates).

Therefore, doses of 1.8 and 0.85 mM of SA in tomato plants under water deficit caused a greater reduction of *Ci*, in the years 2019 and 2020, respectively, at 15 DWD (Figure 3A). Comparing with the plants of the control treatment, in 2019, the application of SA reduced the *Ci* values at the level of plants without water restriction, while, in 2020, the control plants showed higher *Ci* when compared to the plants in which the aforementioned SA dose was applied. At 30 DWD (Figure 3C), the same dose (1.3 mM of SA) was efficient in the reduction of *Ci* in the two years studied. They also showed similar values of this variable when compared to the control plants. For the last evaluation, at 45 DWD (Figure 3E), the dose of 0.8 mM of SA promoted the greatest decrease in *Ci* values in the two studied years. The *Ci* values were similar between the control plants and the tomato plants that received this dose of SA in water restriction.

With the increase in stomatal conductance, an increase in transpiration of tomato plants treated with SA under water deficit was also observed (Figure 3 B, C, F). At 15 DWD (Figure 3B), higher values of E were observed at doses of 1.4 and 0.9 mM of SA in the years of 2019 and 2020, respectively. In 2019, the *E* of the plants treated with the mentioned dose was similar to that of the control plants, while, in 2020, the plants that received 100% Etc presented a higher *E* than the others. At 30 DWD (Figure 3D), the dose of 0.75 mM of SA was the one that provided the highest *E* in both years. In 2019, plants treated with the aforementioned dose showed *E* superior to the control group, and in 2020, the control and SA-treated plants showed similar values.

For the evaluation at 45 DWD (Figure 3F), the dose of 0.9 mM of SA was responsible for the largest increase in *E* in the both years of experimentation. In 2019, the application of this dose caused higher values than the control treatment, and in 2020, similar values.

The WUE (Figure 4 A, C, E), ratio between *A* and *E*, was higher when the dose of 1.61 and 2.0 mM of SA was applied in 2019 and 2020, respectively, at 15 DWD (Figure 4A). In 2019, the control plants showed a higher *WUE* when compared to the water deficit with SA application. In 2020, the *WUE* of the plants at the same doses was higher. At 30 DWD (Figure 4C), similar behavior was observed in both years: the dose of 2.0 mM of SA provided higher values of this relationship in tomato plants under water restriction, also higher than those observed in control plants. For the last assessment, at 45 DWD (Figure 4E), the 0.9 and 0.3 mM doses of SA increased the

WUE in 2019 and 2020, respectively. In 2019, the *WUE* was similar to that of the control plants, while in 2020, it was higher.

Another important relationship is the *A*/*Ci*, because through this ratio, it is possible to infer the efficiency of the Rubisco enzyme. The application of SA also positively influenced the carboxylation efficiency (Figure 4B,D,F). At 15 DWD (Figure 4B), doses of 1.5 and 1.1 mM of SA provided higher *A/Ci* values in 2019 and 2020, respectively. For both years, those doses promoted an increase in this variable to values similar to those of plants without water deficit. At 30 DWD (Figure 4D), the dose of 1.3 mM of SA was the one that promoted the greatest increases in *A*/*Ci*, in the two years of cultivation, to the point of being superior to the control plants. At 45 DWD (Figure 4F), the doses of 0.9 and 0.3 mM of SA provided higher *A*/*Ci* values in 2019 and 2020, respectively. In 2019, the *A*/*Ci* was similar between the control plants and those that received the mentioned dose. In 2020, tomato plants treated with the dose of SA showed higher values than the control group.

Positive effects caused by SA on gas exchange of tomato plants under water deficit, especially in CO_2 assimilation and carboxylation efficiency, were reflected in plant production. Total production (Figure 5A) reached its maximum with the dose of 1.1 mM of SA in 2019, while in 2020, the dose of 1.6 mM of SA promoted the greatest response. In 2019, the control plants showed higher production, without significantly differing from the plants treated with SA under water deficit. While in 2020, the application of the aforementioned dose increased tomato production to the level of control plants that did not undergo water restriction.

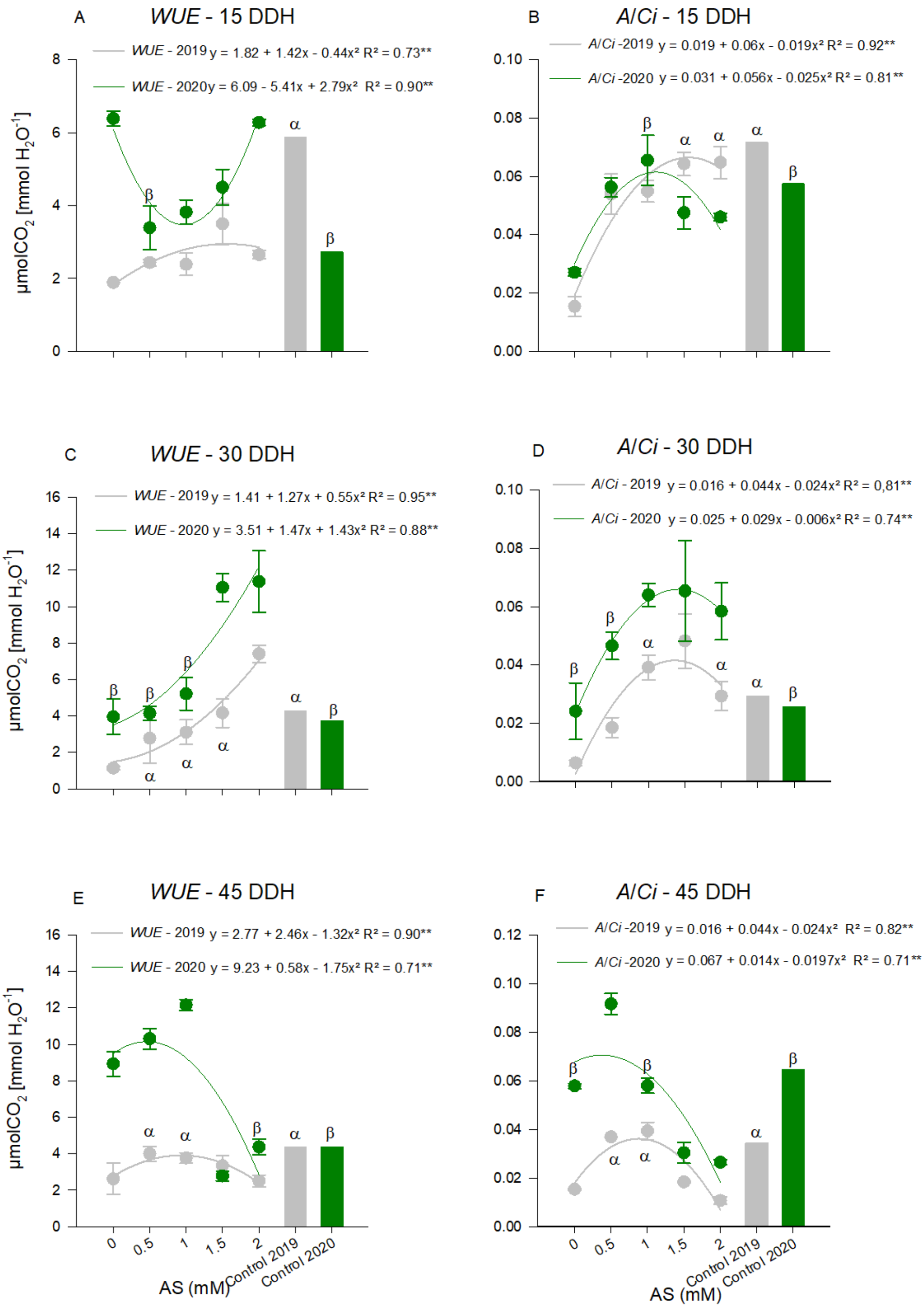

Figure 4. Water use efficiency (*WUE)* at 15 (**A**), 30 (**C**), and 45 (**E**) days of water deficit (DWD); carboxylation efficiency (*A*/*Ci*) at 15 (**B**), 30 (**D**), and 45 (**F**) DWD of tomato plants submitted to the application of salicylic acid (SA) doses in two consecutive years (2019 and 2020). $^{\alpha}$ Indicates an equal and significant effect between treatments by Dunnett's Test at 5% probability in the 2019 cycle and $^{\beta}$ in the 2020 cycle. The bars show the standard deviation. *n* = 4 (number of replicates).

2.3.2 Productive characteristics

For commercial production (Figure 5B), in 2019, the dose of 0.95 mM of SA provided the highest production, as well as favoring the production of commercial fruits. It can be observed that plants subject to water deficit and application of SA presented commercial fruit production similar to those of plants that did not suffer water restriction. For the 2020 cycle, the dose of 1.3 mM of SA increased the commercial fruit production of plants under water deficit and also presented values similar to those of plants that were not under water restriction.

The application of SA was also efficient in increasing the number of fruits per plant. This is probably due to the observed reduction in floral abortion, which increases the number of total and commercial fruits. For TNF (Figure 5C), in 2019, the dose of 0.5 mM of SA was efficient in maintaining the flowers until fruit formation, which is why the plants of this treatment presented TNF similar to the control plants. In 2020, the dose of 1.1 mM of SA was efficient in maintaining the flowers, however, it did not reach the values of the control plants. The same behavior of TNF was observed for CNF (Figure 5D), in 2019. The dose of 0.5 mM of SA was the most efficient and provided a number of commercial fruits similar to that of the control plants. In 2020, the dose of 1.8 mM of SA in plants under water deficit promoted higher CNF, without, however, showing similarity to the control plants.

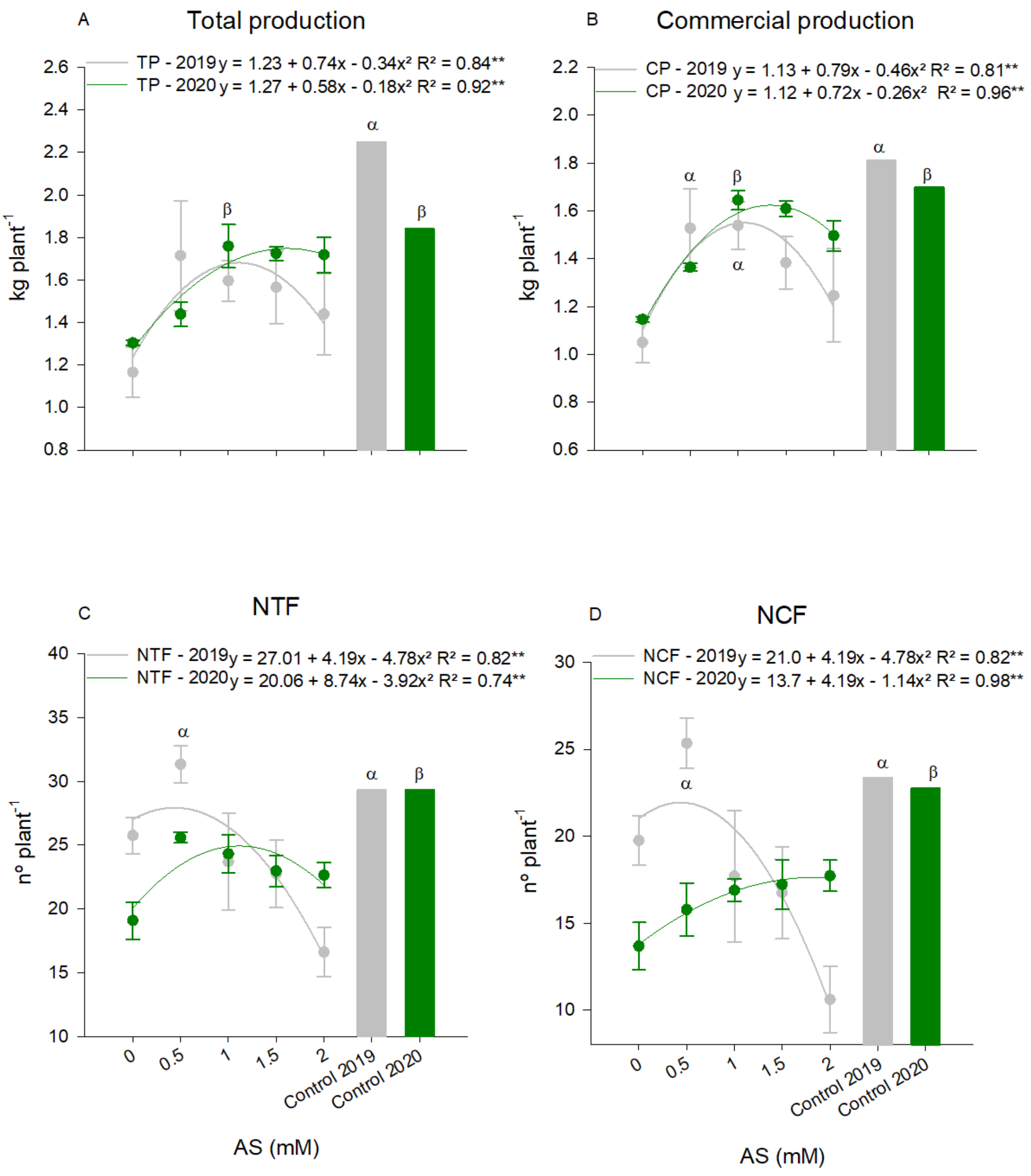

Figure 5. Total production (**A**), commercial production (**B**), total number of fruits (TNF) (**C**), and commercial number of fruits (CNF) (**D**) of tomato plants submitted to the application of doses of salicylic acid (SA) and water deficit in two consecutive years (2019 and 2020). $^{\alpha}$ Indicates an equal and significant effect between treatments by Dunnett's Test at 5% probability in the 2019 cycle and $^{\beta}$ in the 2020 cycle. The bars show the standard deviation. *n*= 4 (number of replicates).

2.4 DISCUSSION

Our results suggested that the foliar application of 1.3 mM of salicylic acid increased by 55% the *A*, 58% the A/Ci of tomato plants under water deficit, causing a reduction in floral abortion, which represents about 25% more of total and commercial fruits. With the increase of *A* and *A*/*Ci*, the photoassimilates may have been translocated to the fruits, resulting in a 30% increase in the commercial production of fruits in plants treated with AS under water deficit.

During their life cycle, plants are exposed to unfavourable conditions that cause stress and these can be of biotic or abiotic origin [27]. Water deficit is a major abiotic stress of the latter kind and that inhibit plant metabolism [28]. One of the first plant responses to water deficit is stomatal closure, which results in reduced photosynthesis [29]. In transgenic tomato plants, results indicated that in significant stressful situations, like water deficit or phosphorus deficiency for example, glycinebetaine increased content can inhibit the accumulation of ROS and/or act on the activation of the plasma membrane HC-ATPase which can enhance the transport of phosphorus. However, non transgeninc plants cannot count on this strategy, since this tomato plants are not able to accumulate GB under both normal and stress conditions [30,31]. In this way, there is a reduction in the parameters of *A*, g_s, and *E*, with the decrease in water availability in the soil. This relationship was observed in tomato plants under water deficit without SA application. Similar behaviour was recognized in ryegrass under water deficit conditions [32].

Nevertheless, the dose of 1.4 mM of SA promoted a higher g_s in tomato under water deficit and, consequently, increased *A* values. At certain moments of the evaluation, the assimilation of CO_2 and stomatal conductance of plants under water deficit and treated with SA was similar to those of plants of the control group, indicating that this plant regulator can control the stomatal closure caused by water deficit.

At 30 e 45 DWD in 2020, no differences were found in *A* and g_s between control and water deficit without SA application; in fact, the water defect was imposed because the gs was low in the treatments without SA and under deficit, while the control plants were under environmental stress (high temperature, high VPD, low humidity), and the SA, in turn, was able to alleviate water deficit and environmental stress in tomato plants. 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. Similarly, Sohag et al. [33] confirms that the application of SA might activate plant defensive system and helped plant to adjust the water status under drought, due to the alleviation of drought-induced over-accumulation of ROS, possibly by enhancing the activities of antioxidant enzymes. Also, SA improved g_s which increased *A*, that is, greater relation of *A*/*Ci*; consequently, reflecting on plant height and weight accumulation in fruits [34].

Plant regulators can lead to water deficit tolerance by modifying biochemical and physiological processes, such as maintaining stomatal opening. Although a complex process, the stomatal movement has abscisic acid (ABA) as an important regulatory component to govern It in response to reduced water availability [35]. It also stimulates stomatal closure through secondary messengers, such as reactive oxygen species (ROS), nitric oxide, calcium, and protein kinases [36]. The application of SA, in turn, reduced the content of ABA and ethylene, resulting in higher stomatal conductance and photosynthesis of mustard plants under water deficit [37].

The hormonal balance is modified in plants under water deficit, promoting an increase in ethylene and ABA concentrations as an adaptation strategy to reducing water availability. Samui et al. (2020) reported that an alternate wetting and drying situation in the soil can be conducive for increased ABA concentration in xylem sap of tomato plants [38]. However, at the same time that it works to protect plants from water deficit, it can compromise photosynthesis and plant growth. As a management strategy for crops, foliar application of SA plays a key role in reducing the ethylene and ABA content [37]. Thus, it is interesting to observe that under water deficit conditions, foliar application of SA increased CO_2 assimilation, stomatal conductance, and water use efficiency of tomato plants, possibly by minimizing the stress caused by ROS that reduced the formation of ethylene and ABA.

Similarly, to the behaviour of g_s, tomato plants that received a foliar application of SA of about 1.0 mM showed higher *E*. Transpiration is closely related to the water status of the plant, probably due to better water absorption. Thanks to the accumulation of osmotic substances, such as soluble sugars and proline [39]. The increase in this variable is also important to regulate leaf temperature and, thus, favor the action of metabolic processes such as photosynthesis. Hence, Richards et al. (2002) stated that stable production requires high transpiration, stomatal conductance, and mesophilic conductance [40].

In cultivation conditions without water deficit, plants show high transpiration due to the maintenance of CO_2 input through the stomata, a fact observed in tomato plants without water restriction that showed high *E*. In this context, the foliar application of SA on tomato plants under water deficit also increased *E*, which maintained the leaf temperature adjusted for the functioning of the Rubisco enzyme, a fact observed with the increase of *A*/*Ci*, which boosted total and commercial production numbers.

The lower *A*/*Ci* in tomato plants under water deficit and without SA may be related to changes in any biochemical reactions or changes in thylakoid membrane composition caused by water deficit [41] since *Ci* was high in these plants. Thus, the supply of CO_2 to Rubisco was not compromised. The reduction in carboxylation efficiency may be linked to the non-functioning of metabolic and enzymatic processes essential for *A*, which was reduced in the plants that received SA treatment. Within this context, the role of SA in increasing the *A*/*Ci* of plants under water deficit may be linked to the prevention of auxin oxidation [42], whose high content increases CO_2 assimilation in the leaf. The application of SA also enhances the action of the carbonic anhydrase (CA) enzyme [43], which facilitates the diffusion of CO_2 through the chloroplast membrane, catalyzing the hydration of dissolved CO_2 as it enters the lower stromal alkaline environment [44]. CA also catalyzes the reversible hydration of CO_2 and maintains a constant supply of CO_2 for the Rubisco enzyme.

Other researches also showed that *A*/*Ci* was closely associated with SA application, demonstrating a positive regulatory role in increasing CO_2 fixation in corn plants under saline stress [45].

Salicylic acid can also affect on flowering in a variety of plants, increasing the permanence of flowers in the plant and regulating flowering time. It can also act in defense of the plant, seeking reproductive development [17,46]. The exogenous application of 1.0 mM of SA increased the number of inflorescences of marigolds (*Calendula officinalis* L.) [47]. Under water deficit conditions, it was not possible to observe an inducing effect on tomato flowering. However, the foliar application of this plant regulator resulted in lower floral abortion in tomato plants, which resulted in increases in the total and commercial numbers (TNF and CNF) of fruits. Tomato plants from the control group, which grew in a field condition without water deficit, presented the expected TNF and CNF. In contrast, plants under water deficit and without application of SA showed lower values for both due to floral abortion caused by water restriction. The application of SA reduced the stress of the water deficit and minimized the fall of the flowers, increasing the accumulation of photo-assimilates in the fruits that, resulted in enhancement in the total and commercial production of fruits.

In this sense, it has been reported that SA affects improving photosynthetic capacity due to the stimulation of the Rubisco enzyme and the increase in photosynthetic pigments [48]. This statement corroborates the results of this research where the carboxylation efficiency (*A*/*Ci*), which shows the activity of the Rubisco enzyme, increased about 60% in plants treated with SA compared to plants without SA treatment and under water deficit. The increase in *A* and *A*/*Ci* resulted in boosts in total and commercial tomato fruit production, around 40 and 45%, respectively. In field conditions, without water deficit, according to other researchers, the foliar application of 0.05 mM of SA increased the production of tomato fruits [49], and the dose of 0.1 mM was efficient to increase cucumber production [50].

The effectiveness of exogenous SA application depends on the plant species, stage of development, applied concentration, application method, and environmental conditions [51,52]. Thus, for the use of SA in plants under water deficit, the application of higher doses of this plant regulator is important to mitigate the lack of water effects.

Adjusting photosynthetic capacity under water restriction is vital for plant survival. Tomato plants under water deficit presented the modulation of gas exchange as a strategy, such as the reduction of stomatal conductance (g_s) and transpiration (*E*), which resulted in lower assimilation of CO_2 (*A*). On the other hand, foliar application of SA resulted in an increase in g_s and *E* and, consequently, in *A*. We also observed an effect on *A*/*Ci*, which increased in tomato plants treated with SA. This increase resulted in a better distribution of photo-assimilates to flowers and fruit. Thus, floral abortion was reduced, and the fruits accumulated mass (Figure 6).

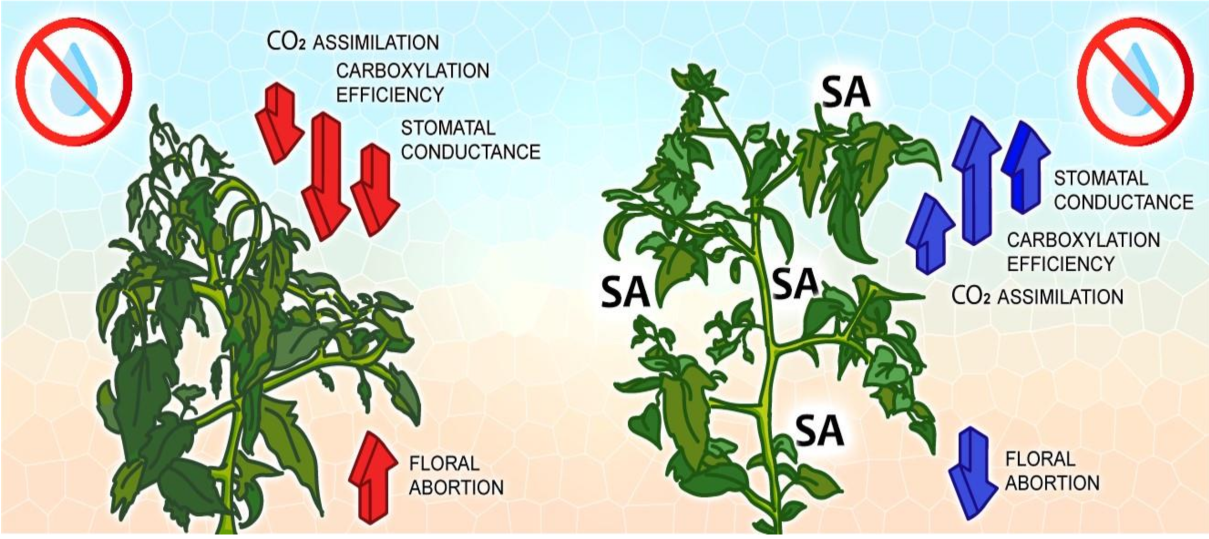

Figure 6. Graphical abstract of Foliar application of salicylic acid to mitigate water stress in tomato

According to our results, foliar application of SA is a technique capable of mitigating the deleterious effects of water deficit on gas exchange and tomato production. Hence, it can be used to manage this tomato in regions with low water availability

2.5 CONCLUSIONS

With our results, we can conclude that tomato plants showed to be sensitive to water deficit, with a reduction in gas exchange and fruit production when only 70% of ETc was replaced. However, the foliar salicylic acid application was efficient in mitigating the adverse effects caused by water deficit on gas exchange and tomato fruit production under field conditions

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CONSIDERAÇÕES FINAIS

A aplicação foliar de ácido salicílico em plantas de tomateiro sob estresse hídrico ou não, desencadeia uma série de respostas bioquímicas e fisiológicas, como a ativação das enzimas antioxidantes SOD, CAT e POD, aumento do conteúdo de prolina, aumento da condutância estomática e, consequentemente, da assimilação de CO_2.

Em condição de ambiente protegido nossos resultados indicam a aplicação foliar de AS; Pois a partir da inclusão dessa técnica no manejo da cultura ocorre a intensificação do sistema antioxidante da planta, ativando as enzimas SOD, CAT, POD e, consequentemente, reduzindo a peroxidação lipídica. Com a manutenção da integridade da membrana celular, o aparato fotossintético das plantas fica protegido, o que aumenta a eficiência do PSII das plantas tratadas com AS. Este regulador vegetal também aumenta o acúmulo de prolina, um importante osmorregulador, garantindo altos *gs* e *A* e aumentando os fotoassimilados. O conjunto dessas melhorias acarreta na maior produção comercial e total de frutos.

Para condição de restrição hídrica em campo, os resultados também são promissores. Podemos destacar que a aplicação foliar de AS promoveu a assimilação de CO_2 e condutância estomática em plantas com défice hídrico semelhante às plantas sem restrição hídrica. A condutância estomática é mantida mesmo com suprimento de água reduzido em plantas tratadas com AS. Na produção, a aplicação foliar AS reduz o aborto floral e direciona os fotoassimilados para os frutos, o que aumenta a produtividade do tomateiro sob défice hídrico.

Assim, a aplicação foliar de AS é eficiente em mitigar os efeitos deletérios da deficiência hídrica em plantas de tomateiro quanto às trocas gasosas e produção de frutos. A aplicação foliar deste regulador vegetal pode ser indicada para condições de campo em regiões com baixa disponibilidade hídrica ou défice hídrico relacionado ao clima.

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