BASIC AREAS
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Exogenous arginine modulates leaf antioxidant enzymes and hydrogen peroxide content in tomato plants under transient heat stresses

Vivyan Justi Conceição[1], Simone Costa Mello[1,*], Marcia Eugenia Amaral Carvalho[2], Salete Aparecida Gaziola[2], Ricardo Antunes Azevedo[2]

1. Universidade de São Paulo – Escola Superior de Agricultura "Luiz de Queiroz" – Departamento de Produção Vegetal – Piracicaba (SP), Brazil.
2. Universidade de São Paulo – Escola Superior de Agricultura "Luiz de Queiroz" – Departamento de Genética – Piracicaba (SP), Brazil.

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***Corresponding author:** scmello@usp.br

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ABSTRACT: Heat stress can impact crop development and yield and amino acids play diverse essential roles in plants. This work aimed to study the long-term effects of foliar spray with L-arginine in antioxidant machinery, physiology, nutrition, productivity and fruit quality of tomato plants subjected to transient heat stresses. Six concentrations of L-arginine were sprayed on the plants: 0 (control), 0.10, 0.25, 0.50, 1.0 and 2.0 $g \cdot L^{-1}$. The content of hydrogen peroxide (H_2O_2), a reactive oxygen species, decreased concurrently to the increasing arginine concentration. The ascorbate peroxide (APX) activity had an inverse behavior to that observed for H_2O_2 content ($r = -0.79$), not only indicating that arginine is able to modulate APX, but also suggesting that this enzyme plays an important role on the mitigation of H_2O_2 generation under heat stress. Ascorbate peroxide and catalase (CAT) activities had a positive correlation ($r = 0.82$), showing that these enzymes may work *in tandem*. The influence of arginine on photosynthesis activity and gas exchange was generally weak and depended mainly on the plant developmental stage. Yield was increased by 19.8 and 23.1% in plants that received 1.0 and 0.5 $g \cdot L^{-1}$ of arginine, respectively, when compared to control plants. In conclusion, the use of exogenous L-arginine can protect tomato plants against oxidative imbalance under transient heat within protected environments.

Key words: amino acids, antioxidant machinery, ascorbate peroxidase, catalase, oxidative stress, *Solanum lycopersicum*.

The world demand for tomato (*Solanum lycopersicum* L.) fruits rises every year due to their multiple utilizations (FAOSTAT 2020), that include *in natura* consumption and production of processed sauces and therapeutic compounds (Bergougnoux 2014). Therefore, several growers are using protected environments in order to mitigate effects of biotic and abiotic stressors on plants and, consequently, to improve tomato yield. However, some abiotic factors are still hard to manage even in protected environments during the summer. For instance, heat stress can be detected in plants from regions with hot seasons (Lang et al. 2020), despite the use of ventilators and water sprays (Ferrari and Leal 2015).

High temperature inhibits photosynthetic activity, alters cellular homeostasis, impairs growth of vegetative and reproductive organs and accelerates plant physiological maturity, frequently triggering reductions in the crop productivity while increasing fruit disorders and visual damages (Lang et al. 2020; Nagarajan and Nagarajan 2010; Singh et al. 2017; Wang et al. 2018). Most of such side effects are resulted from oxidative stress, which arises from a disproportion between production and elimination of reactive oxygen species (ROS) that can trigger protein oxidation, cytotoxicity and even DNA abandonment, hence threating the cellular viability (Soares et al. 2019).

In order to maintain the cell redox homeostasis, plants activate a powerful and multifaceted antioxidant system that is composed by enzymatic and nonenzymatic components. The enzyme superoxide dismutase (SOD) acts as the first line of plant cell defense, dismutating O_2^- to H_2O_2 that can be subsequently scavenged by the antioxidant enzymes guaiacol peroxidase

(GPX), catalase (CAT) and ascorbate peroxidase (APX) (Soares et al. 2019). Bearing in mind the importance of the mitigation of oxidative stress to reduce crop losses, researchers and growers are using natural and/or artificial compounds that are able to enhance the plant antioxidant system, such as seaweed extracts and amino acids (Carvalho et al. 2018; Serciloto et al. 2014).

The use of amino acid-based products for alleviation of effects from high temperatures is not a new approach, but data originated from long-time application of amino acids are scarce. Therefore, this study aimed to evaluate the influence of foliar spray with different concentrations of the amino acid L-arginine on the antioxidant machinery, physiology, nutritional status, productivity and physicochemical features of fruits from tomato plants, which were grown in a protected environment, after their exposure to transient heat stresses. The hypothesis is that application of foliar sprays containing L-arginine on tomato plants could enhance responses of their antioxidant machinery against transitory heat stresses and, consequently, improve the fruit quality and yield in such conditions.

Seedlings of tomato cultivar Pizzadoro were used in the experiment carried out in a greenhouse located at Piracicaba, São Paulo, Brazil. In this region, the climate is Cwa, according to the Köppen's classification (i.e., subtropical climate with dry months in winter — July to August — and rains in summer). During period of crop cultivation, the average temperature inside the greenhouse was 25 °C, reaching peaks of 35.1 °C (Fig. 1). The photosynthetic active radiation (PAR) ranged between 26.51 and 28.18 mol·m^{-2}·day^{-1} with average value of 27.60 mol·m^{-2}·day^{-1}. The average, maximum and minimum relative humidity was 77, 98 and 42%, respectively.

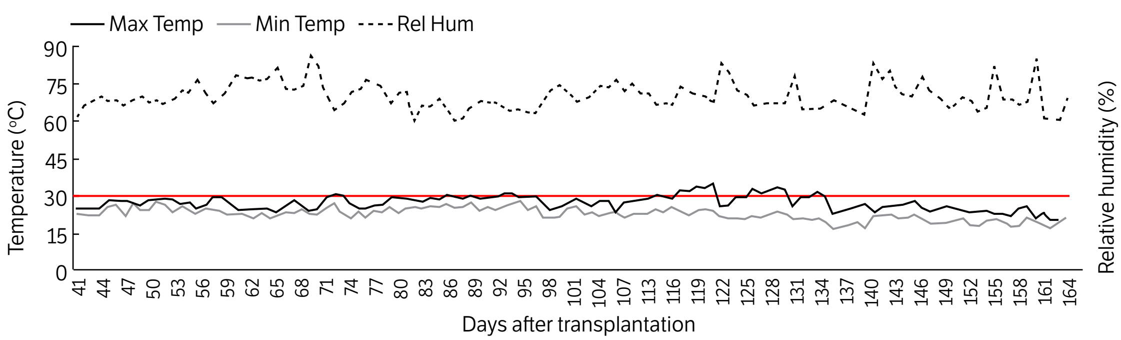

Figure 1. Average air relative humidity, and maximum and minimum temperatures during the experimental period.

Note. Red line indicates 30 °C, a temperature from which the side effects caused by heat on plant development and yield are potentially enhanced. Relative humidity = rel hum, maximum temperature = max temp and minimum temperature = min temp.

Six concentrations of L-arginine were applied through foliar spray (0, 0.1, 0.25, 0.50, 1.0 and 2.0 g·L^{-1}), which was performed every 15 days from the beginning of blooming stage on the 21st, 39th, 59th, 79th and 99th days after transplantation (DAT) of seedlings. The volume used for sprays ranged from 500 to 1200 L·ha^{-1}, according to the plant development. Plants were grown in pots filled with coconut fiber (Golden Mix, Amafibra). An automatized fertigation system (Irrigas), based on tensiometers, was used to control water and nutrient supply to the plants, according to their developmental stage. In the first growth period (from seedling transplantation to maturation of the first bunch), solution 1 was used in the fertigation system; afterwards, it was replaced by solution 2 (Table 1).

The net photosynthesis rate (A) and stomatal conductance (g_s) were evaluated three days after application of arginine treatments. The evaluations were performed from 3 to 5 pm in a completely expanded leaf from one plant of each replication on the 38th and 52nd DAT of seedlings by using portable gas exchange system equipment (model LI-6400XT).

On the 40th DAT, nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), sulfur (S), boron (B), copper (Cu), iron (Fe), manganese (Mn) and zinc (Zn) concentrations were evaluated according to methodology of Malavolta et al. (1997). The fourth newly expanded leaf was collected from four plants that were used to estimate an average value in each of the three replications from each treatment.

Malondialdehyde (MDA) and hydrogen peroxide (H_2O_2) contents, as well as the activities of SOD (EC 1.15.1.1), CAT (EC 1.11.1.6) and APX (EC 1.11.1.11) were analyzed in the newly completely expanded leaves. In the greenhouse, the leaves were harvested on the 62nd DAT and placed in liquid nitrogen. Next, all samples were stored in a −80 °C freezer until analyses. Leaf tissues were grinded to a fine powder in liquid nitrogen before the analysis onset.

Table 1. Nutritive solutions used for cultivation of tomato (*S. lycopersicum* 'Pizzadoro') plants during their vegetative and reproductive stages (solutions 1 and 2, respectively).

Fertilizer	Solution 1 ($mg \cdot L^{-1}$)	Solution 2 ($mg \cdot L^{-1}$)
Calcium nitrate	600	750
Potassium nitrate	0	0
Potassium sulphate	400	574
Magnesium sulphate	375	640
Monoammonium phosphate	0	0
Monopotassium phosphate	281.25	224.4
Magnesium nitrate	37.5	0
Ammonium nitrate	125	0
Iron (6.5%)	3	3
Boric acid	5	5
Conmicros*	25	25

* Composition (%): B (1.82); CuEDTA (1.82); FeEDTA (7.26); MnEDTA (1.82); Mo (0.36); Ni (0.36); ZnEDTA (0.73).

Table 2. Pearson's correlation analysis among variables related to the activity of catalase (CAT), superoxide dismutase (SOD) and ascorbate peroxidase (APX); content of hydrogen peroxidase (H_2O_2) and malondialdehyde (MDA); fresh weight of commercial fruits (CF); and the rates of net photosynthesis (A) and stomatal conductance (g_s) in tomato (*S. lycopersicum* 'Pizzadoro') plants treated six L-arginine concentrations (0, 0.1, 0.25, 0.50, 1.0 and 2.0 $g \cdot L^{-1}$).

	CAT	SOD	APX	H_2O_2	MDA	SF	MF	LF	CF	*A*	g_s
CAT	-	0.72*	0.82***	-0.51*	-0.09	0.02	-0.28	0.11	-0.18	-0.34	-0.31
SOD		-	0.68***	-0.27	0.26	-0.04	-0.08	-0.10	-0.10	0.07	-0.06
APX			-	-0.79***	-0.05	0.02	-0.14	0.11	-0.07	-0.17	-0.20
H2O2				-	0.04	-0.09	-0.14	-0.11	-0.17	0.25	0.20
MDA					-	-0.03	0.28	-0.18	0.15	0.37	0.16
SF						-	0.20	0.29	0.65**	0.20	0.29
MF							-	0.15	0.85***	0.29	0.20
LF								-	0.43	-0.28	-0.35
CF									-	0.25	0.21
A										-	0.91***
gs											-

***, **, * significant at 0.1, 1 and 5%, respectively.

Lipid peroxidation was measured as MDA content according to Heath and Packer (1968), and H_2O_2 content was determined as described by Alexieva et al. (2001). Protein content was determined by using bovine serum albumin as standard (Bradford 1976). The extraction of antioxidant enzymes was carried out according to Azevedo et al. (1998). Superoxide dismutase total activity was determined according to procedure of Cembrowska-Lech et al. (2015). Catalase activities were quantified by spectrophotometer, as described by Azevedo et al. (1998). Ascorbate peroxidase activity was analyzed following procedures of Nakano and Asada (1981).

Fruit harvests were performed from February 21 (DAT 61) to April 17 2017 (DAT 111). The total fruit production was the sum of both commercial and noncommercial fruits. For determination of commercial fruit production, the weight of small (50–65 mm), medium (66–80 mm) and large-sized (81–100 mm) fruits was taken into account.

Fruits with both less than 50 mm of diameter and visual damages (such as cracks and stains) were considered as noncommercial fruits. The total soluble solids (TSS), pulp pH, total titratable acid (TTA), TSS/TTA ratio and ascorbic acid content were determined in six fruits of each experimental unit at DAT 93 (Carvalho et al. 1990).

The experimental design was randomized blocks with six treatments [0 (control), 0.1, 0.25, 0.50, 1.0 and 2.0 $g{\cdot}L^{-1}$ of arginine] that contained three replications with 10 plants per plot, from which only the six central potted plants were used for statistical analyses. The assumptions for the analysis of variance (ANOVA, i.e., normal distribution, variance homogeneity and error independence) were checked for every variable. Next, data were subjected to ANOVA ($p \leq 0.05$) and means were compared by Duncan test ($\alpha \leq 0.05$) by using the statistical analysis system (SAS 2011) software. In addition, the Pearson's correlation analysis was employed to evaluate relations among some of the studied variables.

The optimum temperature for tomato plant development varies from 21 to 26 °C, depended on crop stage (Rivero et al. 2004; Sadashiva et al. 2016). However, temperatures next to 30 °C were frequently detected in different stages of tomato development, reaching peaks of 35.1 °C (Fig. 1), so potentially increasing the occurrence of negative effects on pollen quality, fruit set and/or ripening (Jegadeesan et al. 2018; Singh et al. 2017). Most of these side effects are linked to oxidative stress, which is triggered by the increased generation of dangerous compounds, such as ROS, to the biological macromolecules (Frank et al. 2009; Soares et al. 2019). According to Wang et al. (2018), high temperature causes oxidative burst of superoxide anion and/or H_2O_2 in plants due to the inhibition of energy and electron transference in the photosystem II.

In order to cope with exacerbated ROS generation, tomato plants are able to modulate antioxidant machinery by enhancing the content of nonenzymatic antioxidants, such as carotenoids and sugars (Carvalho et al. 2020a; Zhou et al. 2017), and improving the activity of antioxidant enzymes like SOD, CAT and APX (Frank et al. 2009; Soares et al. 2019). An enhanced APX activity, which was related to the reduction of H_2O_2 content, was observed by increasing L-arginine concentration sprayed on plants (Fig. 2 and Table 2). This result not only suggests that exogenous arginine is able to modulate APX activity, but also indicates that arginine-driven APX activity plays an important role in the mitigation of H_2O_2 generation in tomato plants subjected to transient heat stresses. An inverse relation between H_2O_2 content and APX activity was also observed in arginine-treated tomato plants subjected to drought (Nasibi et al. 2011), reinforcing evidences that exogenous arginine can affect the performance of APX activity.

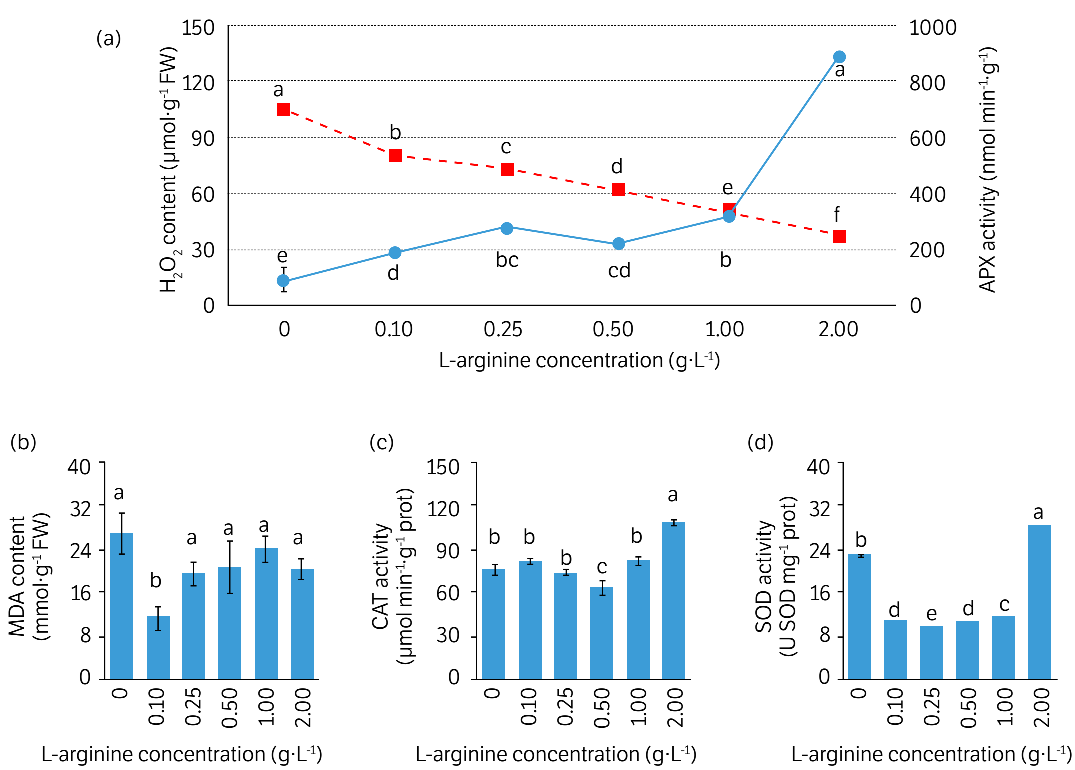

Figure 2. Oxidative stress indicators and activity of antioxidant enzymes in tomato leaves.

Note. a) hydrogen peroxide content (H2O2) and ascorbate peroxidase activity (APX); b) malondialdehyde content (MDA); c) catalase activity (CAT); d) superoxide dismutase activity (SOD) in leaves of tomato (*S. lycopersicum* 'Pizzadoro') plants that received foliar spray with different L–arginine concentrations. Squares represent H2O2 content, and circles represent APX activity. Means followed by different letters, for each variable, differ by Duncan's test ($p \leq 0.05$) ($n = 3$).

Data from the present study also indicate that APX and CAT activities may act *in tandem* in arginine-treated plants to mitigate ROS production (Fig. 2, Table 2). According to Wang et al. (2018), a diverse ROS-scavenging network functions in concert in chloroplasts, including mainly APX-glutathione cycle, to support the equilibrium between ROS generation and scavenging. One hypothesis to explain these plant responses may be linked to the action of nitric oxide synthase-like enzymes (that use arginine as substrate), which induce post-translational modifications in antioxidant enzymes, such as CAT, SOD and monodehydroascorbate reductase (MDHAR) (Corpas et al. 2019). In addition, it is known that arginine is a precursor of polyamines (PAs), which are potential scavengers of different ROS, like $^{\bullet}OH$ and $^1O_2$ (Das and Misra 2004).

The PAs have multiple roles in plant development, likely due to their ability to regulate DNA replication, transcription and translation; cell proliferation; enzyme activities; cellular cation-anion balance and membrane stability (Gill and Tuteja 2010). Because PAs synthesis is increased in plants facing environmental challenges, and the use of different approaches to enhance the production PAs also lead to an improved plant tolerance to stresses (Chen et al. 2019), PAs are considered natural alleviatory agents of the effects from nonoptimal environmental conditions on plants (Spormann et al. 2021). For instance, the foliar application of PAs on two wheat varieties under heat stress decreased both grain damages and MDA content while enhancing the activity of antioxidant enzymes, such as SOD and CAT (Jing et al. 2020). Modifications in APX activity also occur simultaneously to alterations in PAs content and type, evidencing close relation between PAs and antioxidant enzymes (Spormann et al. 2021). However, with the current data, the mechanism by which arginine modulates antioxidant machinery cannot be clearly established.

The foliar spray of arginine neither improved plant nutritional status (Table 3) nor changed the pH, TSS, TTA, TSS/TTA ratio and ascorbic acid content in fruits (data not shown). The effect of arginine on tomato physiology seems to be stronger in younger plants, since they were more responsible on the 38th than 52nd DAT. In this younger stage, the net photosynthesis and stomata conductance rates reached the lowest values at 0.1 $g \cdot L^{-1}$, while the other arginine concentrations provoked no alterations in relation to the control plants (Fig. 3). No significant differences were observed among treatments for plant productivity, despite increases by 23.1 and 19.8% in tomato yield after the application of this amino acid at 0.5 and 1.0 $g \cdot L^{-1}$ (Fig. 4). This increment can be agronomically and commercially important for growers, but further studies using large-scale tomato production should be carried out. It is also important to take into consideration the role of nutrients in eventual plant protective mechanisms to neutralize the side effects of abiotic stress (Carvalho et al. 2020b), but no major difference was noticed in treated plants, when compared to the control ones, for the concentration of essential elements (Table 3).

Table 3. Nutrients in the leaves of tomato (*S. lycopersicum* 'Pizzadoro') plants that received foliar sprays with different L-arginine concentrations (0, 0.1, 0.25, 0.50, 1.0 and 2.0 $g \cdot L^{-1}$).

Arginine	N	P	K	Ca	Mg	S	Cu	Fe	Zn	Mn	B
0	45.50a	5.96a	25.90a	17.06a	2.87a	5.83a	49.33a	120a	58.00a	234.66a	43.33ab
0.1	45.50a	6.10a	26.86a	17.40a	3.10a	6.06a	38.00a	128a	61.33a	239.33a	45.66ab
0.25	46.66a	6.00a	27.40a	17.10a	3.00a	6.23a	36.00a	129a	60.33a	237.33a	43.66ab
0.50	45.96a	5.93a	27.90a	17.66a	3.16a	7.80a	50.66a	149a	66.66a	243.33a	56.66a
1.00	43.86a	6.43a	25.36a	16.40a	3.67a	5.83a	58.00a	136a	56.00a	240.00a	38.00b
2.00	45.50a	6.23a	25.36a	17.23a	3.01a	5.60a	54.00a	140a	58.33a	241.33a	40.00ab

Distinct letters denote different means by Duncan test ($p \leq 0.05$) for comparison among treatments.

Overall, this study demonstrated that exogenous L-arginine can affect the performance of antioxidant enzymes and the content of ROS in tomato plants subjected to transient heat stresses within protected environment. However, these results should be validated in field trials, since the better understanding on the mechanisms that support plant response to challenging environments is necessary to guide both crop management and biotechnological programs for food security purposes in times of fast environmental changes.

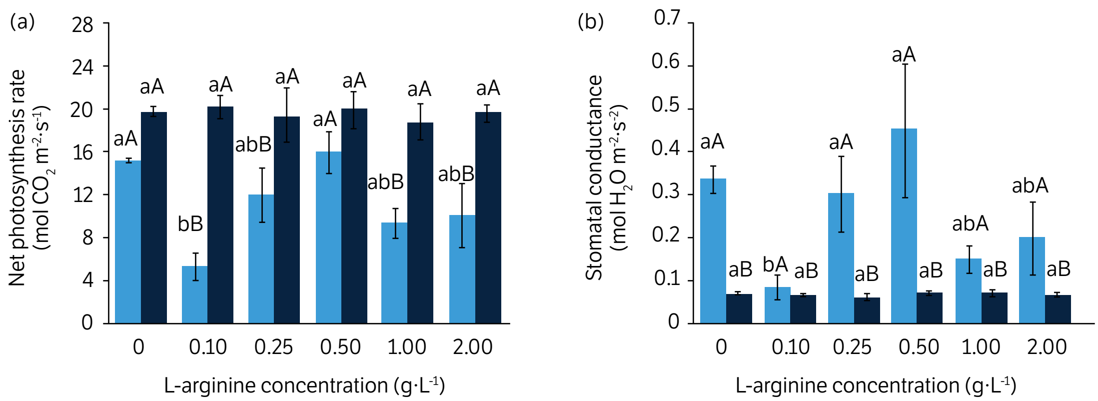

Figure 3. Net photosynthesis rate and stomatal conductance in leaves of tomato (*S. lycopersicum* 'Pizzadoro') plants that received foliar spray with different arginine concentrations.

Note. Distinct lowercase and uppercase letters denote different means by Duncan's test (α≤ 0.05) for comparison of different arginine concentrations in same period of plant stage, and for comparisons between plant stages for a same arginine concentration, respectively. White and black columns represent evaluations that were performed on the 38th and 52nd DAT of tomato seedlings to pots, respectively (n = 3).

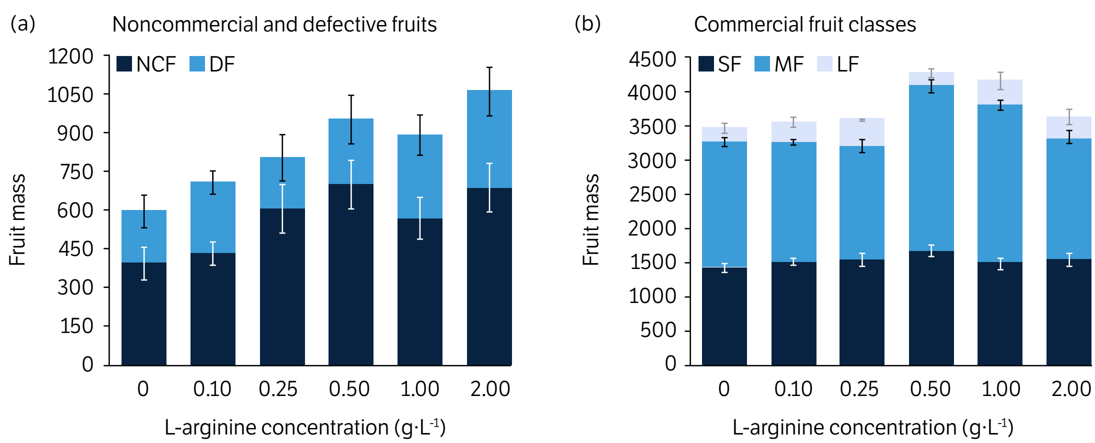

Figure 4. Fresh mass of noncommercial, defective fruits and classes of commercial fruits in tomato (*S. lycopersicum* 'Pizzadoro') plants that received foliar spray with different arginine concentrations (n = 3).

Note. NCF = noncommercial, DF = defective fruits; classes of commercial fruits: SF = small, MF = median and LF = large.

AUTHORS' CONTRIBUTION

Conceptualization: Mello S. C.; **Formal analysis:** Carvalho M. E. A. and Conceição V. J.; **Methodology:** Mello S. C.; **Investigation:** Conceição V. J.; **Writing – Original Draft:** Carvalho M. E. A.; **Visualization:** Carvalho M. E. A.; **Writing – Review and Editing:** Conceição V. J., Gaziola S. and Azevedo R. A.; **Funding Acquisition:** Mello S. C.; **Resources:** Mello S. C. and Azevedo R. A.; **Supervision:** Mello S. C.

DATA AVAILABILITY STATEMENT

All dataset were generated or analyzed in the current study.

FUNDING

Not applicable.

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