Research Article

Variations in Bioactive Content in Different Tomato Trusses due to Elicitor Effects

Luciano Ávila-Juárez and Herminio Miranda-Rodríguez

Biosystems Engineering Group, Biosystems Laboratory, Faculty of Engineering, Autonomous University of Queretaro, CU Cerro de las Campanas, S/N, Colonia Las Campanas, 76010 Santiago de Queretaro, QRO, Mexico

Correspondence should be addressed to Luciano Ávila-Juárez; lucianoavila.j@hotmail.com

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The tomato fruit is rich in bioactive compounds with antioxidant activity, the levels of which can vary over time in response to biotic and abiotic factors, including the application of elicitors. We investigated the effects of foliar spray of methyl jasmonate (MeJ), salicylic acid (SA), nitric oxide (NO), and hydrogen peroxide (H_2O_2) on tomato plants every 15 days until the end of cultivation. We measured the levels of bioactive compounds, antioxidant activity, and physiological parameters in three distinct trusses. With the exception of plant length, the elicitors had no effects on physiological parameters, whereas they did have an effect on lycopene content, bioactive compound levels, and antioxidant activity in the three sampled trusses. A strong correlation between bioactive compounds and antioxidant activity was found for the elicitors, particularly MeJ. Our results indicate that certain bioactive compounds and their antioxidant activities vary not only between trusses but also based on the specific elicitor used.

1. Introduction

The tomato is one of the most widely grown fruits worldwide, known for its economic significance and nutritional properties. The tomato has been intensively studied due to its high levels of molecules with antioxidant activity (AA), such as carotenoids, phenols, and flavonoids [1, 2]. Indeed, the tomato has been named a functional food [3] because some of its bioactive compounds (BCs) with AA could play protective roles in reducing chronic diseases, such as cancer and cardiovascular disease [4]. In some diets, tomato consumption represents the primary source of lycopene (71.6%), the secondary source of β -carotene (17.2%), and the tertiary source of vitamin C (6.0%) [5]. However, the levels of BCs in the plant can vary based on genetic, physiological, and agronomic factors [6–8]. Thus, fruits from different trusses may not have the same attributes unless they are grown in tightly controlled environments with respect to abiotic and biotic factors. For example, environmental factors affect the AA [9], UV light induces the accumulation of flavonoids and other phenols [10], and lycopene synthesis is severely inhibited by intense solar radiation and high temperatures [9, 11]. Some

studies have shown monthly variability in the AA and in the content of phenol compounds, lycopene, and β -carotene, in the tomato fruit [12, 13]. Exogenous application of elicitors to plants has been used to increase the BC content in the fruit. Salicylic acid (SA), methyl jasmonate (MeJ) [14], hydrogen peroxide (H_2O_2), and nitric oxide (NO) [15] are molecules known to elicit a wide variety of plants, such as alfalfa, broccoli, tomato, and pepper, and these molecules act by inducing the expression of genes to activate BC biosynthesis [14]. However, the plant will respond according to the nature, doses, frequency of application, and place of implementation of the elicitor, possibly activating some BCs but deleting others [14] or even affecting the basic functions of primary metabolism, such as photosynthesis [16].

To the best of our knowledge, no information is available concerning the levels of antioxidants or BCs in trusses of different ages following elicitor application. Most research has focused on chemical analysis from harvests or collections of fruit (pepper and tomato) from trusses of different ages [15, 17, 18].

Therefore, the objective of this work was to assess the production of specific BCs, antioxidant activities, and quality

parameters in fruits at three different time points (truss: 1, 5, and 9) following the application of elicitors (SA, MeJ, NO, and H_2O_2) in tomato plants.

2. Materials and Methods

2.1. Plant Material, Cultivation Conditions, and Treatments. Tomato seeds (*Solanum lycopersicum* L. cv. Rafaello from Ahern, USA) were planted in polystyrene germination trays with 50 cavities with vermiculite and peat moss and placed in a germination chamber (80% RH, 25°C) until germination. Once the plants sprouted, they were hydroponically grown in Rockwool slabs (1 m $\times$ 0.2 m $\times$ 0.075 m) in a greenhouse (1000 m²) with a density of 3 plants·m⁻². The greenhouse was located in Amealco, Queretaro state, Mexico (20° 11'17" N latitude and 100° 08'38" W longitude, altitude 2629 m). The nutrient solution used [19] included the following components (mg·L⁻¹): N (167), P (31), K (277), Mg (49), Ca (183), S (111), Fe (1.33), Mn (0.62), B (0.44), Cu (0.02), Zn (0.11), and Mo (0.048), prepared at a pH of 5.8. The electrical conductivities of the nutrient solutions were as follows: 1.5 dS·m⁻¹ for the third transplant to the truss, 2 dS·m⁻¹ from the third truss to the sixth truss, and 2.5 dS·m⁻¹ from the sixth truss until the end of cycle [20]. The amount of irrigation by plant was in line with the development of the crop and was always monitored until 25% of the water drained. The temperature, relative humidity, and radiation total fluctuated between 29.4/10.5°C (day/night), 52.4/31.7% (day/night), and 933 W·m⁻² (maximum), respectively, during the experiment. An experimental design was used with random complete blocks with three replicates per treatment and three plants per replicate. Five treatments in total were implemented according to the type of elicitor used (SA, MeJ, NO, H_2O_2 , and a control).

2.2. Elicitors Used. SA, H_2O_2 , sodium nitroprusside (SNP; NO donor), and MeJ were purchased from Sigma-Aldrich (St. Louis, MO, USA). The doses and forms of the elicitor preparation were as follows: 0.5 mM SA [21], 0.5 mM MeJ [22] (storage in 10% methanol), 5 mM H_2O_2 [23], and 100 mM SNP [24]. In both cases, distilled water was used to prepare the elicitors, and the pH was adjusted with KOH (1.0 N) to 6.5. At 15-day intervals, five times in total, the first five leaves from the apical meristem of each plant were doused with 100 mL per plant; this treatment was performed in the morning (8–9 a.m.). The control plants were sprayed with distilled water.

2.3. Fruit Samples. At each harvest, tomato samples were taken from three batches of nine tomatoes. The external color was measured near the equatorial diameter using a Minolta Chroma Meter (CR 400; Minolta Osaka, Japan) with the CIE $L^*a^*b^*$ color space. Only fruit in the network ripe stage with similar external appearances were selected. The samples were cut into slices and then frozen in liquid nitrogen and stored at –80°C prior to analysis.

2.4. Quantification of Carotenoids. To analyze lycopene and β -carotene levels, we selected the first, fifth, and ninth trusses. Lycopene and β -carotene (both from Sigma-Aldrich) were

extracted from the tomato fruit and quantified according to Ávila-Juárez et al. [20]. Briefly, 1 mL of 2.5% butyl hydroxytoluene (BHT) was added to 50 mL of the homogenized tomato. The mixture was shaken for 10 min. Then, 20 mL of hexane:acetone:ethanol (2:1:1, v:v:v) was added, and the mixture was shaken again and filtered through a glass Büchner funnel. Next, 10 mL of hexane was added and shaken, and the upper layer was collected and dried; the residue was analyzed to identify the carotenoids. Lycopene and β -carotene were detected at 450 nm and 290 nm, respectively. The carotenoid composition and concentration were determined by HPLC using a YMC reversed-phase carotenoid column (Merck, USA) (C[30]; 150 $\times$ 4.6 mm, i.e.; S-[3] μ m) with an isocratic eluent of 55% methanol, 40% acetonitrile, 4% dichloromethane, and 0.1% BHT. The results are expressed as mg·kg⁻¹ of fresh fruit (FW).

2.5. Quantification of Total Phenols and Flavonoids. Samples were homogenized in a blender (Ultra Turrax) for 1 min and protected from light with aluminum foil. The homogenized samples (1 g) were extracted with 10 mL of aqueous methanol and filtered through 0.45 μ m Durapore paper. The filtrate was stirred (Orbit 1000 Labnet, USA) for 24 hours in the dark at room temperature. Then, the samples were centrifuged at 5000 rpm for 10 min at 4°C. The supernatant was recovered and stored in the dark at –20°C prior to analysis.

Total phenols were extracted using the Folin-Ciocalteu colorimetric method according to Dewanto et al. [25]. Briefly, tomato extract samples (40 μ L) were diluted with 460 μ L of distilled water. Then, 250 μ L of Folin-Ciocalteu solution was added. The samples were mixed well and allowed to stand for 10 min prior to the addition of 1250 μ L of a 20% sodium carbonate aqueous solution. Then, the samples were allowed to stand for 120 min in the dark at room temperature prior to measurement at 760 nm versus the blank using a MULTI-SKAN GO microplate spectrophotometer (Model 5119300; Thermo Scientific, Vantaa, Finland) compared with the standard. A standard with a known gallic acid concentration was used for curve calibration (0.0–160 μ g gallic acid·mL⁻¹). All values are expressed as the mean of three replicates in micrograms of gallic acid equivalent (GAE) per gram of tomato.

The method for the determination of the flavonoid content described previously [26] was modified for use with microplates. Briefly, 50 μ L of the methanolic extract was mixed with 180 μ L of distilled water and 20 μ L of a 1% 2-aminoethyldiphenylborate solution. The absorbance of the solution was monitored at 404 nm with the MULTISKAN GO microplate spectrophotometer (Model 5119300; Thermo Scientific, Vantaa, Finland). Finally, the extract adsorption was compared with the adsorption of the rutin standard at concentrations ranging from 0.0 to 200 μ g·mL⁻¹. All flavonoid content values are expressed as the mean of three replicates in milligrams of rutin equivalent (RE) per gram of tomato.

2.6. Antioxidant Activity in the 1,1-Diphenyl-[2]-picrylhydrazyl (DPPH) Radical Inhibition Assay. The radical scavenging capacity (antioxidant activity) analyzed by the DPPH method for tomato extracts was adapted for use with microplates

TABLE 1: Average physiological variables measured in the fruit and tomato plant throughout the experiment.

Elicitor applied	Plant				Fruit		
	Yield (kg pl ⁻¹)	Length (m pl ⁻¹)	Diameter (mm)	Fresh weight (g fruit ⁻¹)	Diameter (mm)	°Brix	pH
SA	7.67	3.81 ^a	11.07	153.46	57.71	4.77	4.63
MeJ	7.18	3.35 ^b	10.74	143.78	56.31	4.59	4.63
NO	7.34	3.58 ^{ab}	10.97	146.82	56.68	4.79	4.64
H ₂ O ₂	7.48	3.59 ^{ab}	10.98	149.74	56.97	4.91	4.65
Control	7.31	3.48 ^{ab}	10.47	146.20	56.66	4.83	4.64
ANOVA	ns	*	ns	ns	ns	ns	ns

* Different letters in the same column represent significant differences at $P \leq 0.05$ and “ns” represents not significant. NO: nitric oxide, SA: salicylic acid, MeJ: methyl jasmonate, and H₂O₂: hydrogen peroxide. Means = 10 trusses.

according to Oomah et al. [26]. Briefly, a DPPH solution (150 μ M) was prepared in 80% aqueous methanol. The samples or the standard (20 μ L) was added to wells in a 96-well flat-bottom visible light plate containing 200 μ L of DPPH solution. The plate was covered and left in the dark at room temperature. Then, the absorbance was measured at 520 nm in the MULTISKAN GO microplate spectrophotometer (Model 51119300; Thermo Scientific, Vantaa, Finland) at 30, 60, 90, and 120 min. For each sample or standard, a 100 μ M BHT solution (20 μ L) was added to a well in a 96-well flat-bottom visible light plate containing 200 μ L of DPPH solution. The water-soluble vitamin E analogue Trolox (6-hydroxy-[2,5,7,8]-tetramethylchroman-[2]-carboxylic acid) was used to prepare the standard curve (range, 0.0–800 μ M). The activity is reported as micromolar Trolox equivalent antioxidant activity (TEAC) per 100 grams of sample.

2.7. Statistical Analysis. The data were analyzed using analysis of variance (ANOVA). When a significant difference was detected, the means were compared using the Tukey test (with $P \leq 0.05$) in Origin Pro 8.0 (OriginLab®, USA).

3. Results

3.1. Variation in Bioactive Compounds between Treatments. No significant differences were found ($P \leq 0.05$) in the physiological variables with the exception of plant length. SA resulted in a greater plant length (3.81 m·pl⁻¹) than the MeJ application (3.35 m·pl⁻¹) (Table 1).

The CIE color space values in the fruits subjected to the different treatments in the various trusses were similar (sampled $P \leq 0.05$), with the following means: $L^* = 40.1$; $a^* = 29.3$; $b^* = 23.5$; chroma and hue = 41.4. The plants elicited with SA and H₂O₂ showed 112 and 73% more lycopene, respectively, in the tomato fruit in the first truss compared with the control ($P \leq 0.01$). The coefficient of variation (CV) for the lycopene content following SA treatment was the second lowest after the control (CV = 20.7). There were no significant differences in the levels of β -carotene, total phenols (TP), or flavonoids in any of the sampled trusses with the elicitors used here. However, treatment with H₂O₂ resulted in a greater CV for β -carotene, whereas SA treatment showed a CV of 61.6 for TP and NO treatment showed a CV of 54.7 for the flavonoids. Significant differences ($P \leq 0.05$) in

AA were detected in truss five between treatments, with MeJ eliciting the lowest AA (69.83 μ M TEAC·100 g⁻¹ FW) with the highest CV (CV = 56.1) (Table 2).

3.2. Variations in Bioactive Compounds between Trusses. Significant differences in BC levels were found between the sampled trusses. For example, lycopene was equal in trusses one and nine following SA application, whereas truss five presented the lowest value for this variable (0.96 mg·100 g⁻¹ FW). For this same treatment, significant differences ($P \leq 0.05$) were detected for AA between the first and ninth trusses, with a rising trend similar to that seen in the controls. For the NO applications, significant differences were found ($P \leq 0.05$) in TP (59.52 μ g GAE·g⁻¹ FW) and flavonoids (51.99 mg RE·mg⁻¹ FW) for the first truss compared with the ninth, with a downward trend (Table 2). MeJ treatment resulted in a significant difference ($P \leq 0.05$) for AA between the first and ninth trusses, the latter of which was higher than the first (292.8 μ M TEAC·100 g⁻¹ FW). In contrast, for this same treatment, the lycopene, β -carotene, TP, and flavonoid levels were equal in the three trusses. A similar trend was observed for the H₂O₂ treatment, in which TP, flavonoid, and β -carotene levels remained similar, but AA was significantly ($P \leq 0.05$) higher in truss nine (229.36 μ M TEAC·100 g⁻¹ FW). In this same treatment, significant differences were found for lycopene content between the trusses, with a lower value for the fifth truss (0.86 mg·100 g⁻¹ FW) compared with the ninth and first trusses (Figure 1). With respect to the levels of BCs in the control, significant differences ($P \leq 0.05$) were found for lycopene, TP, and AA. The highest AA and lycopene values were found in truss nine (233.56 μ M TEAC·100 g⁻¹ FW and 1.60 mg·100 g⁻¹ FW, resp.) relative to truss one, with a rising trend. By contrast, we found significantly lower levels ($P \leq 0.05$) of TP (15.56 μ g GAE·g⁻¹ FW) in truss nine, with a descending trend (Figure 2).

The relationships between BCs in the trusses in each treatment were evaluated via Pearson's correlation. We found a negative relationship ($r = -0.6903$, $P < 0.05$) between β -carotene levels and AA following SA treatment, and we observed the most relationships between BC levels and AA for the MeJ treatment. Only one relationship was identified between TP and flavonoids for the NO treatment ($r = 0.8076$, $P < 0.05$). A relationship between lycopene and AA was identified in the control (Table 3).

TABLE 2: Behavior^a of carotenoids, flavonoids, and phenols and total antioxidant activity as a result of the application of elicitors in tomato.

Elicitor applied	Lycopene (mg 100 g ⁻¹ FW)			β -Carotene (mg 100 g ⁻¹ FW)			Total phenols (μ g GAE g ⁻¹ FW)			Flavonoids (mg RE g ⁻¹ FW)			Antioxidant activity (μ M TEAC 100 g ⁻¹ FW)		
	1 ^b	5 ^b	9 ^b	1 ^b	5 ^b	9 ^b	1 ^b	5 ^b	9 ^b	1 ^b	5 ^b	9 ^b	1 ^b	5 ^b	9 ^b
SA	2.74 ^a	0.96	1.76	22.1	1.85	1.36	0.81	29.9	60.93	48.68	20.74	61.6	24.71	25.26	18.62
MeJ	1.37 ^b	0.92	0.86	29.6	0.70	1.59	0.76	58.5	51.69	26.73	43.52	34.4	35.90	14.03	39.72
NO	1.72 ^{bc}	1.08	0.99	40.8	1.46	0.95	1.24	36.9	59.52	35.09	27.41	41.1	51.99	27.14	16.16
H ₂ O ₂	2.24 ^{ac}	0.86	1.45	31.8	2.09	0.83	0.73	78.1	42.25	40.77	28.74	32.5	26.73	30.65	25.22
Control	1.29 ^b	1.22	1.60	20.7	0.63	1.52	0.93	50.1	54.11	26.41	15.56	58.7	26.70	25.50	31.97
ANOVA	**	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	*	ns

^a Average of three replicates. CV represents the coefficient of variation of the mean values. b represents the number of sampled trusses. Different letters in the same column represent significant differences at * $P \leq 0.05$ and ** $P \leq 0.01$ based on Tukey's test. ns: not significant.

FIGURE 1: Behavior of bioactive compounds and antioxidant activity in three different trusses, and the effects of elicitor application on tomato plants. Each point is the average of three replicates. Different letters between rows indicate significant differences at $P \leq 0.05$. Units for lycopene: $100 \text{ g mg}^{-1} \text{ FW}$; β -carotene: $100 \text{ g mg}^{-1} \text{ FW}$; phenols: $\mu\text{g GAE g}^{-1} \text{ FW}$; flavonoids: $\text{RE g mg}^{-1} \text{ FW}$; antioxidant activity: $\mu\text{M TEAC } 100 \text{ g}^{-1} \text{ FW}$ (a) SA; (b) MeJ; (c) NO; (d) H_2O_2).

FIGURE 2: Behavior of bioactive compounds and antioxidant activity in three distinct trusses in the control group. Each point is the average of three replicates. Different letters between rows indicate significant differences at $P \leq 0.05$. Units for lycopene: $100 \text{ g mg}^{-1} \text{ FW}$; β -carotene: $100 \text{ g mg}^{-1} \text{ FW}$; total phenols: $\mu\text{g GAE g}^{-1} \text{ FW}$; flavonoids: $\text{RE g mg}^{-1} \text{ FW}$; antioxidant activity: $\mu\text{M TEAC } 100 \text{ g}^{-1} \text{ FW}$.

TABLE 3: Pearson's correlation (r) at a $P \leq 0.05$ significance level with linear regression on the treatments for different antioxidants and secondary metabolites.

Treatment	Evaluation	r	P
SA	β -Carotene-antioxidant activity	-0.6903	0.0395*
MeJ	β -Carotene-total phenols	-0.6976	0.0367*
MeJ	Total phenols-flavonoids	0.8784	0.0018**
MeJ	Flavonoids-antioxidant activity	0.8339	0.0052**
NO	Total phenols-flavonoids	0.8076	0.0084**
Control	Lycopene-antioxidant activity	0.7460	0.0210*

* and ** indicate significance at $P \leq 0.05$ or $P \leq 0.01$, respectively.

4. Discussion

SA has positive effects on physiological processes, such as the stem diameter and yield of the tomato fruit [21]. In the results presented here, the SA applications resulted in a significant increase in the plant length, but the findings did not agree with Yildirim and Dursun [21], who found a

better performance in the tomato fruit with the same dose (0.05 mM). However, soil was used as a means of growth in that investigation, whereas an inert substrate was used here; thus, the variation could have been affected by the growth environment rather than the elicitor.

4.1. Behavior of Bioactive Compounds between Treatments. Antioxidant compounds (ACs), such as the carotenes, flavonoids, and phenolic compounds contained in tomato fruits, have been linked to decreased risks of cancer and heart disease [27, 28]. The levels of these ACs can be increased using elicitors such as SA, MeJ, and NO, which have been found to activate genes related to BC synthesis [29]. However, BC composition also varies in response to factors such as the time of year and environmental conditions [13]. To avoid the harvest of fruits in different states of maturity, the a^*/b^* maturation index was used [30], which showed no significant differences between the treatments ($P < 0.05$) for the L^* , a^* , b^* , chroma, and hue values. The mean values (a^*/b^*) for the three sampled trusses were 1.27 (first truss), 1.26 (fifth truss), and 1.25 (ninth truss), which were within the range reported by [12].

There is a close relationship between SA and H_2O_2 because H_2O_2 is involved in the synthesis of SA, whereas the conversion of benzoic acid in SA is catalyzed by H_2O_2 through benzoic acid 2-hydroxylase activation, with the application of one inducing the other [31]. SA acts as a phytohormone that regulates the synthesis of photosynthetic pigments [32] and increases BCs [33], whereas H_2O_2 acts as a second messenger to regulate the expression of genes involved in the synthesis of antioxidant enzymes [34]. Based on these results, both SA and H_2O_2 significantly increased lycopene levels in the first truss, with a CV (22.1% and 31.8%, resp.) similar to that of the control (CV = 20.7). These results are consistent with those of Raffo et al. [12], who reported a CV of lycopene of 20.1 in different months of the year, and with the range for lycopene reported by Martínez-Valverde et al. [35].

According to Smith [36], the synthesis of phenolic compounds can be influenced by light. Light increases the activity of enzymes, especially phenylalanine ammonia lyase (PAL), which is involved in the synthesis of phenolic compounds. The amount of light received during the experiment most likely did not affect the flavonoid and TP contents because no significant differences were found between the treatments used here.

An antioxidant is any substance that can prevent the oxidation of cellular components even when present in low concentrations [37]. AA is influenced by the degree of maturity of the fruit [12], temperature, and radiation [13]. SNP is widely used as a NO donor in plants to study the effects of NO [38]; when implemented in low quantities, SNP acts as an intra- and intercellular messenger involved in the regulation of various biochemical processes [39] and increases AA in vitro [40, 41] as well as the levels of some BCs [42]. Based on our results, significant differences were found in AA for the NO applications in truss five relative to the other treatments, except the control.

4.2. Variations in Bioactive Compounds between Trusses. The variables β -carotene, flavonoids, and TP were equal in the three trusses sampled in the AS, MeJ, and H_2O_2 treatment groups. Although genetic control represents the main factor that determines the accumulation of phenol compounds in vegetables, external factors can modify the contents [43]. We detected a significant decrease in TP levels in truss nine with respect to the first truss in the control, with a downward trend. By contrast, only flavonoid and β -carotene levels were equal in the three trusses. Following treatment with NO and MeJ, significant differences were found for lycopene content in the three trusses, with a descending trend. In the control, we detected significant differences in lycopene content, which was 24% higher in the ninth truss compared with the first. Regarding lycopene content, our results are similar to those reported by Raffo et al. [12], who found variations in the monthly lycopene contents in tomato fruit. Few data have been reported in the literature on the variation of BCs in tomatoes, much less as the result of an elicitor. Significant differences were found in TP and flavonoids between the first and ninth trusses following the application of NO, with the behavior of these two variables decreasing. By contrast, the lycopene, β -carotene and AA levels were equal in the three trusses, suggesting that similar values might be obtained for these variables in different crops in response to NO.

Next, we analyzed AA behavior between the trusses in each treatment. In general, with the exception of NO, AA levels increased in all treatments, particularly SA, the control, and H_2O_2 . H_2O_2 (1.0 mM) and SA (0.5 mM) applications in tomato plants were found to increase AA in the fruit [44]. The increases in AA were likely due to the implementation of elicitors during the early stages of the plant, which had an effect on the subsequently sampled trusses in the SA and H_2O_2 treatments, whereas the MeJ and NO treatments led to different effects in the three sampled trusses. More research is needed on this topic because measured AA levels can be affected by other components involved in the complex antioxidant system in the tomato fruit [45]. Furthermore, the effect of the elicitor on any BC depends not only on the dose but also on the age of the plant [46].

4.3. Mapping between AA and Bioactive Compounds. The relationship between the different antioxidants and BCs in each treatment was assessed through the Pearson correlation. The results showed that SA applications in tomato plants caused a negative correlation between the amount of β -carotene and the AA. In contrast, a positive correlation was detected between lycopene and the AA in the control treatment, as was reported in other investigations [47–49]. The antioxidant capacity (lipophilic antioxidant activity) of a fruit is provided by the carotenes (mainly lycopene and, in small amounts, β -carotene) [50]. Both the SA and H_2O_2 treatments resulted in increased lycopene content, although no correlations with AA were found.

Several authors have studied the correlation between BCs and AAs in tomatoes [48, 51, 52] and found a positive correlation independent of the method used to determine the AA. For example, Hdider et al. [53] found a positive correlation of $r = 0.426$ ($P < 0.01$) between the lycopene content

and the AA using the 2,2'-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) method. Our results show a correlation of $r = 0.74$ ($P < 0.05$) in the control treatment with the DPPH test. In contrast, the negative correlation found between β -carotene and AA might have been due to metabolite degradation during the analysis [54] because β -carotene showed a downward behavior among the three trusses sampled in most treatments. According to the correlation analysis, a positive correlation ($P < 0.01$) between TP and the flavonoid content and between the flavonoid content and AA was found following the MeJ applications. These results are in agreement with those of Hdider et al. [53], who reported a positive correlation between the flavonoids and AA. Flavonoids confer benefits to tomato antioxidants as a result of their power to remove free radicals [55]. This finding suggests that the AA should increase with the flavonoid content in the fruit as demonstrated in this study because the AA is mainly influenced by hydrophilic antioxidants (83%), such as polyphenols [56].

A positive correlation between the TP and flavonoids was found following treatment with both NO and MeJ. Various works have demonstrated the presence of polyphenols in tomato. Indeed, the AA can be estimated with both flavonoids and TP [2]. Thus, a close correlation between these compounds and AA would be expected, as found by García-Valverde et al. [50], consistent with the results of this investigation ($r = 0.807$, $P < 0.01$). MeJ activates enzymes responsible for the biosynthesis of polyphenols, such as phenylalanine, from which both TP and flavonoids are derived [57].

In conclusion, the application of elicitors in tomato plants did not have significant effects on fruit yield. However, there were significant variations in levels of BCs in different trusses from the same plant. The above results indicate that bioactivity is not constant in the fruit but is dependent on several factors. Elicitation techniques may represent a technique for maintaining adequate bioactive content in tomato fruit based on the truss being harvested.

Conflicts of Interest

The authors declare no conflicts of interest.

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