Improvement of the nutraceutical quality and yield of tomato by application of salicylic acid

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Abstract

Tomato fruits are a unique functional food and a natural reservoir of nutrients, antioxidants, vitamins and bioactive compounds that improve nutrition and human health. As an important vegetable throughout the world, tomatoes have attracted the attention of researchers to carry out various strategies to improve the functionality of this food aimed at the prevention of diseases, health and global well-being. An agronomic strategy in this study was to evaluate the influence of the application of “salicylic acid (SA)” on the yield and nutraceutical quality of the tomato crop, produced under hydroponic conditions. A completely randomized experimental design with six repetitions was used. SA at five doses (0.025, 0.05, 0.075, 0.1 and 0.125 mM) and one control were applied every 15 days in the nutritive solution under a hydroponic system on tomato plants. The evaluated variables were yield (total fruit weight per plant), fruit parameters (weight, diameter, firmness, and total soluble solids), percentage of weight loss and nutraceutical quality of tomato. The results obtained indicate that the addition of salicylic acid in nutritive solution increased the yield and biosynthesis of phytochemical compounds in tomato fruits, in relation to the control without application. In conclusion, to obtain a higher nutraceutical quality without affecting the tomato fruit yield, it is recommended to use the average concentration (0.125 mM) of SA.

Keywords: biostimulation; elicitors; human health; nutrient solution; phytochemicals

Introduction

Tomato (Solanum lycopersicum L.) is the most cultivated vegetable worldwide, since it is the main ingredient in traditional and processed foods, in addition to the important nutraceutical content that this fruit
contains (Islam et al., 2018). Yields and quality are heterogeneous due to genotypic variations, environmental conditions, production system, pests and diseases, which negatively impacts grower’s economy (Pandey et al., 2017; Rai, 2020).

In Mexico, the largest production is centred in the Northwest states, and to a lesser extent in the West (Rios-Osorio et al., 2014). In these areas of the country, tomato production in hydroponic systems under greenhouses has increased markedly in recent years (De la Rosa-Rodriguez et al., 2016). With these cultivation systems, increments in productivity have been achieved, however, the nutraceutical quality of the produce is not always related to these increases (Preciado-Rangel et al., 2018).

Elicitors use is a technique that allows to improve the synthesis of bioactive compounds in vegetables without compromising crop yield (Larqué-Saavedra and Martin-Mex, 2007; Wang et al., 2015; Wen et al., 2019). Salicylic acid (SA) stands out among the most promising elicitors to stimulate crop development and production, which has been shown to induces resistance to any type of stress (Xu et al., 2009), improves development of plants (Yusuf et al., 2013), improves photosynthetic action (Shah et al., 2019), increases yield (Semida et al., 2017) and the shelf life of fruits and the nutraceutical quality (Chen et al., 2016; Ennab et al., 2020).

Therefore, the objective of this research work was to determine the effect of the addition of SA via nutrient solution on the yield, percentage of weight loss and nutraceutical quality of tomato fruits grown under hydroponic conditions.

Materials and Methods

Plant material and growing conditions

The study was carried out in a circular greenhouse, covered with plastic polyethylene and a cooling system, located at the Technological Institute of Torreon, Mexico (24° 30’ and 27° north latitude, 102° 00’ and 104° 40’ west longitude, at an altitude of 1,120 m). Sahel hybrid® tomato seedlings with six true leaves were transplanted into 15 kg capacity black polyethylene plastic pots of 500 gauge, which contained river sand and perlite (80:20) as growing media. The river sand was washed and disinfected with a 5% sodium hypochlorite solution. The pots were placed in a double row, in a staggered arrangement, where a density of four plants per square meter was obtained. A drip irrigation system was used to provide three irrigations per day, with each plant receiving 0.6 L every irrigation event, from transplant to start of flowering and 2.5 to 3.5 L from flowering to harvest. The plants were guided to a single stem and to support them, they were tutored with raffia attached to the upper part of the greenhouse structure. Pollination was performed with an electric brush daily, since the beginning of flowering until the fruit was set.

Experimental design and treatments

The experimental design used was completely randomized with six treatments (0, 0.025, 0.05, 0.075, 0.1 and 0.125 mM of SA), where each concentration had six replicates. SA treatments were applied every 15 days via the nutrient solution (Steiner, 1984), after transplant, obtaining a total of seven applications of SA. The pH and electrical conductivity were maintained at 5.5 and 2.0 dS m⁻¹ respectively. The response variables were yield and its components, percentage of weight loss and nutraceutical quality of the fruit.

Yield

The fruits from each treatment were harvested since the first to the fifth tomato cluster when the fruit presented an intense red colour.
Fruit quality
The quality of the fruit was evaluated in three fruits taken at random from each cluster corresponding to each replicate of the treatments, measuring the average weight of the fruit, the polar and equatorial fruit diameter, firmness of the fruit, weight loss and total soluble solids.

Fruit firmness
It was determined with a penetrometer (Fruit Hardness Tester FHT200), with an 8 mm diameter strut, readings were taken on the opposite sides of the fruit and an average was obtained, the results were expressed in Newton units (N).

Weight loss
Weight loss was determined in a sample of 10 fruits acquired from the last tomato cluster harvested. The fruits, under laboratory environmental conditions, seven days after the harvest its weight loss was determined with a scale (Bapred-3 Rhino brand). The difference with respect to the initial weight was obtained, reporting it in percentage.

Total soluble solids (TSS)
They were evaluated in °Brix, for this, a drop of fruit juice was obtained and the reading was determined with a manual refractometer from 0 to 32% (Master 2311).

Nutraceutical quality of the fruit
Total phenols
They were measured by colorimetry using the Folin-Ciocalteau method, proposed by Singleton et al. (1985). Phenols from a 0.5 g sample were extracted with methanol. 750 µL of Na₂CO₃ at 2% was added in a test tube, followed by the addition of 250 µl at 50% of Folin-Ciocalteau reagent, plus a final volume of 1,375 µL of deionized H₂O, adding 250 µL of enzyme extract. Total phenol results were expressed in mg of gallic acid g⁻¹.

Total flavonoids
Determination was performed following the method of Zhishen et al. (1999). Compounds were extracted with methanol. An amount of 0.5 g was homogenized with 5 mL of methanol. It was centrifuged at 4,000 rpm for 10 min at 4 °C. For the mixture, 250 µL of the aliquot was placed in a test tube, followed by the addition of 75 µL of NaNO₂ and stirred by a vortex. After 5 min 150 µL of AlCl₃ was added; then, a volume of 500 µL of NaOH was added, plus a final volume of 2,025 of H₂O. Absorbance was immediately measured by at 510 nm spectrophotometry. Flavonoids were quantified based on a standard catechin curve.

Antioxidant capacity
It was determined by the method proposed by Hsu et al. (2003), using the free radical 1,1-diphenyl-2-picril-hydracil (DPPH), which has an absorption maximum of 517 nm. The extract was obtained by macerating 1 g of seed in 5 mL at 80% methanol, and then centrifuged at 6,000 rpm for 10 min at a temperature of 4 °C. Afterward 0.5 mL of the extract was taken from the resulting supernatant. Finally, it was mixed with 2.5 L of freshly prepared 0.1 mM DPPH solution, which was incubated for 60 min in the dark and cold. Absorbance was measured by at 517 nm spectrophotometry. The values of the DPPH test were obtained with the formula:

\[
\text{Inhibition percentage} = (1-X) \times 100
\]
\[
X = \frac{A_{517} \text{ samples}}{A_{517} \text{ blank}}
\]

Results are expressed in percent inhibition
Lycopene extraction

It was by the method of Fish et al. (2002) with some modifications. Approximately 1 g of sample (seedless) was placed in test tubes covered with 50 mL PTFE aluminium while on ice. Lycopene extraction solution (39 mL) consisting of hexane, 0.05% (w/v) butylated hydroxytoluene (BHT) in acetone, and 95% ethanol in a 1:1:1 ratio was added to the tubes and stirred for 10 min at 180 rpm. 6 mL of cold distilled water was added to each tube and stirred for an additional 5 min for better separation of polar and nonpolar compounds. The tubes were then removed from the stirring and left for 15 min at room temperature for separation into polar and non-polar layers. The supernatant was placed in new 15 mL aluminium-covered test tubes and kept at -80 °C for other experiments. The absorbance of the supernatant (hexane layer) containing lycopene was read three times using the spectrophotometer at a wavelength of 503 nm. Absolute hexane was used as a blank. The amounts of lycopene in the tissues were estimated using the following formula:

\[
\text{Lycopene (mg kg}^{-1}\text{)} = \frac{x}{y} \times A_{503} \times 3.12
\]

Where \(x\) is the amount of hexane (mL), and the weight of the sample, \(A_{503}\) the absorbance at 503 nm and 3.12 the extinction coefficient.

Vitamin C

It was obtained by means of the titration method proposed by Padayatt et al. (2001). Fresh fruit samples of 10 g were used, which were crushed using 10 mL at 2% of hydrochloric acid, filtered and then in a flask Erlenmeyer was adjusted to 100 mL using distilled water. Subsequently, with 10 mL of the diluted, it was titrated using 2,6 dichlorophenolindophenol (1x10^{-3} N) to determine the content of vitamin C using the formula:

\[
\text{Vit C (mg 100 g FW)} = \frac{(\text{mL 2.6 dichlorophenolindophenol})(0.088)}{(\text{total volume})(100)}/(\text{volume of aliquot})(\text{weight of sample})
\]

Statistical analysis

The results obtained were analysed by analysis of variance and the comparison of means with the Tukey test \((p \leq 0.5)\) using the statistical package SAS, version 9.1 (SAS Institute, 2004).

Results and discussion

Yield

The use of SA modified the yield and its components, the highest yield was in the plants treated with the 0.025 mM dose of SA, being 27.43% higher than the control treatment; this increase is caused by an increase in weight and fruits per plant (Table 1). Wada and Takeno (2013) published similar results indicating that AS improves flowering, hence ensuring more fruits per plant and of greater size and weight, since the application of AS accelerates the cell division of all organs in general (Ahmadi et al., 2014). Preciado-Rangel et al. (2019) reported an increase in performance when using low doses; while high doses decrease it. The plant’s response to SA is concentration-dependent, since at low doses performance is promoted and moderate doses improve fruit quality characteristics and induce resistance to stress, while higher concentrations can cause cell death (Tounekti et al., 2013). Even though this effect was not observed in the results, it is possible that a phytotoxicity threshold has not been reached, since being within this threshold causes stress that the plant cannot control (Hayat et al., 2010).
Table 1. Yield components by effect of the different concentrations of SA in the nutrient solution

| Salicylic acid (mM) | NF   | FW (g)     | PD (mm)  | ED (mm)  | Yield (kg plant⁻¹) |
|---------------------|------|------------|----------|----------|---------------------|
| Control             | 23.0 ± 1.78 b* | 98.1 ± 5.29 b* | 58.8 ± 2.28 b* | 43.4 ± 1.90 d* | 2.26 ± 0.27 b* |
| 0.025               | 25.6 ± 1.86 a  | 112.5 ± 11.67 a | 64.9 ± 5.07 a  | 47.7 ± 1.81 ab  | 2.88 ± 0.34 a  |
| 0.05                | 24.6 ± 1.63 ab | 108.9 ± 7.71 a  | 62.6 ± 5.24 ab | 45.2 ± 2.47 cd  | 2.69 ± 0.34 a  |
| 0.075               | 25.3 ± 1.96 a  | 107.6 ± 8.48 ab | 61.4 ± 4.41 ab | 47.4 ± 2.09a bc | 2.72 ± 0.33 a  |
| 0.1                 | 25.6 ± 1.86 a  | 106.9 ± 9.47 ab | 62.3 ± 3.11 ab | 46.0 ± 2.17 bc  | 2.74 ± 0.23 a  |
| 0.125               | 25.1 ± 2.40 ab | 108.3 ± 8.58 a  | 63.0 ± 4.18 ab | 48.5 ± 1.26 bc  | 2.74 ± 0.41 a  |

*Different letters within each column show significant statistical difference (Tukey, p ≤ 0.05). NF = number of fruits; FW = fruit weight; PD = polar diameter; ED = equatorial diameter.

Fruit quality, firmness and weight loss

The firmness in the fruits confers the capacity to resist blows during their transport or commercialization, in addition of increasing their shelf life. The use of the SA improved firmness and decreased fruit weight loss (Table 2); the firmness in the treated fruits with the 0.125 mM dose of SA was 75.3% higher than those of the control treatment. Similar results were informed by Islam et al. (2018) who reported an increase in firmness of cherry tomato fruits with SA use, an effect attributed to decreased ethylene production and inhibition of the action of the enzymes responsible for cell wall degradation, such as cellulase (CEL), pectin-methyl esterase (PME) and polygalacturonase (PG) that depend on ethylene, the phytohormone responsible for fruit ripening (Asghari and Aghdam, 2010). Fruits with less firmness are susceptible to rapid wilting and dehydration (Moreno-Velázquez et al., 2013), thus causing greater weight loss, which is due to loss of water by perspiration and metabolites by respiration (Sahu et al., 2016); SA produces an inhibitory effect on climacteric respiration and ethylene production, which increases the shelf life and reduces the weight loss of the fruits (Kant and Arora, 2014).

Table 2. Quality of tomato fruits due to the effect of SA in the nutrient solution

| Salicylic acid (mM) | Firmness (N) | Weight loss % |
|---------------------|--------------|---------------|
| Control             | 6.6 ± 0.4 d* | 13.5 ± 3.66 a* |
| 0.025               | 8.1 ± 0.5 c  | 11.8 ± 5.92 b |
| 0.05                | 8.3 ± 0.7 c  | 10.1 ± 2.31 b |
| 0.075               | 9.8 ± 1.0 b  | 7.9 ± 2.7 c   |
| 0.1                 | 9.7 ± 0.8 b  | 8.1 ± 2.47 c  |
| 0.125               | 11.5 ± 1.1 a | 6.1 ± 1.20 d  |

*Different letters within each column show significant statistical difference (Tukey, p ≤ 0.05). N = Newton.

Total soluble solids (TSS)

The TSS content is used commercially as an index of fruit quality because it has a high positive correlation with the sugar content (Li et al., 2016). The results confirm that the use of the SA increases the soluble solids in tomato fruits (Figure 1A). The highest TSS values are obtained with the doses of 0.075 and 0.1 mM, which exceed 21.31% of the fruits compared to the control treatment. The results are consistent with those reported by Islam et al. (2018) and Peyro et al. (2017) who informed a greater accumulation of TSS in fruits exposed to SA. Ahmad et al. (2013) pointed out that the increase in TSS was due to the fact that SA improves the efficiency of the rubisco enzyme and increases the content of chlorophyll, therefore, the rate of photosynthesis increases and this is directly reflected in the accumulation of photo-assimilates in the fruits, increasing the TSS.
Figure 1. Effect of different doses of salicylic acid on the content of total soluble solids (A), total phenols (B), total flavonoids (C), antioxidant capacity (D), lycopene (E) and vitamin C (F) in tomato fruits. *Different letters within each column show significant statistical difference (Tukey, p ≤ 0.05)
Nutraceutical quality of the fruits

Total phenols and flavonoids

With the increment in SA concentration, total flavonoid and phenolic compounds increased (Figure 1B and 1C), obtaining the highest amount of these metabolites with a concentration of 0.125 mM, exceeding the control treatment by 79.5 and 97.36%, respectively. Radwan et al. (2019) mention that SA stimulates the synthesis of phenolic compounds, since the appropriate concentrations causes biochemical stress on cell suspensions and improves activity phenylalanine ammonium lyase (PAL) which is an important enzyme in the synthesis of these metabolites (Kong, 2015). Obtaining foods rich in phenolic compounds is desirable in the food industry, since these compounds delay oxidation and lipids degradation increase the quality of food (Argueta-Solis et al., 2018). Their consumption is beneficial for human health, due to their anti-cancer, anti-inflammatory and antimicrobial characteristics (Rodríguez-Carpena et al., 2011; Saeed et al., 2012; Surh, 2018), in addition to the fact that they tend to fight cardiovascular diseases (Wolfe and Liu, 2007).

Total antioxidant capacity

Antioxidants are substances naturally present in food, which prevent adverse effects of reactive oxygen species on the physiological functions of humans (Wilson et al., 2017). The results showed an 8.0% increase in antioxidant capacity (Figure 1D) in the fruits treated with the 0.125 dose, in relation to the fruits of the control treatment. It has been shown that adequate doses of SA increase the content of bioactive compounds in fruits since the secondary metabolism is activated and increase the synthesis of total antioxidants (Larqué-Saavedra et al., 2010; Mora-Herrera et al., 2011). Hayat et al. (2008) reported that the application of SA improves the activity of antioxidant enzymes such as superoxide dismutase, peroxidase and catalase, which represent the first level of defence in plants.

Lycopene

Lycopene is a carotenoid that is found mainly in tomato, it has antioxidant, anti-inflammatory and chemotherapeutic effects on cardiovascular, neurodegenerative diseases and some types of cancer (Przybylska, 2020). The dose of 0.125 mM of SA improved the concentration of lycopene in fruits (Figure 1E) by 22%, in relation to untreated fruits. The results are consistent with those stated by Javaheri et al. (2012) who reported significant increases in lycopene content in tomato with the use of SA. This quality variable is related to antioxidant capacity, which is well known to increase under stress conditions due to the role it plays in neutralizing oxidizing compounds (Tokunaga et al., 2004).

Vitamin C

Ascorbic acid is the most plentiful water-soluble antioxidant found in plants (Yactayo-Chang et al., 2017) and 91% of the requirements come from the consumption of plant products (Tareen et al., 2012). The use of SA significantly improved the vitamin C content in tomato fruits (Figure 1F), the highest values were obtained with the 0.125 mM dose, exceeding the control by 37.81%. Similar works have reported increases in ascorbic acid in strawberry fruits (Mohamed et al., 2018), tomato (Javaheri et al., 2012), potato (Elwan and El-Hamahmy, 2009) and Granada (Mirdelghan and Ghotbi, 2014) when is treated with SA. This behaviour is due to the fact that SA increases the concentration of sugars, such as glucose, which is a precursor compound of vitamin C, which causes it to accumulate in the plant (Baldet et al., 2013).

Conclusions

The application of salicylic acid through the nutritive solution increased the yield and nutraceutical quality of tomato fruits. The addition of salicylic acid in the nutritive solution is an effective method to
stimulate the synthesis of phytochemical compounds and obtain fruits with higher nutraceutical qualities. It is proposed to increase the concentrations of salicylic acid in nutritive solution to determine the dose where the response is non-linear.

Authors’ Contributions

Conceptualization: OSA, PPR; Methodology: OSA, PPR, ESC; Validation: AMR, JAGF; Formal analysis: MFH, ARD; Investigation: OSA; Data curation: PPR, MFH; Funding acquisition: PPR, ESC; Project administration: PPR, ESC; Writing: OSA, PPR; Review and editing: PPR, JAGF. All authors read and approved the final manuscript.

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Conflicts of Interest

The authors declare that there are no conflicts of interest related to this article.

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