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Exogenous salicylic acid and ferulic acid improve growth, phenolic and carotenoid content in tomato

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Abstract: Salicylic acid (SA) and ferulic acid (FA) are considered phenolic compounds that act as elicitors due to their regulatory functions on plant growth, development, metabolic and physiological responses in plants. The aim of this research was to evaluate the effect of SA and FA on growth, fruit quality and synthesis of secondary metabolites in tomato (*Solanum lycopersicum* cultivar Santa Clara). The experiment was conducted in pots in a greenhouse. The application of SA and FA was performed at concentration of 1.0 mmol L^{-1} alone and in combination, with water treated plants as control. Exogenous application of SA and FA either alone or in combination (SA + FA) resulted in increases in biomass accumulation and chlorophyll contents in tomato plant; and soluble sugar, total polyphenol, flavonoids, lycopene and β-carotene contents in fruits. It was concluded that application of SA and FA resulted in higher production and concentration of secondary compounds in tomato.

1. Introduction

Tomato (*Solanum lycopersicum* L.) belongs to the Solanaceae family and it is considered as low-calorie food. It is a good source of vitamins A, C and E, and mineral salts (Adalid *et al.*, 2010; Kazemi, 2014). In addition to vitamins and minerals, tomato has a high lycopene and β-carotene content, which has antioxidant and anticancer properties. Carotenoids is naturally present in many fruits and vegetables and plays an important role as a functional food when consumed as part of the diet, producing specific health benefits such as reducing the risk of various diseases (Martínez-Hernández *et al.*, 2016; Mehta *et al.*, 2018) and polyphenols (flavonoids, flavanones and flavones) are also present in significant amount in tomato acting as antioxidant, anti-mutagenic, anti-proliferative, anti-inflammatory and anti-atherogenic activities (Martí *et al.*, 2016; Chaudhary *et al.*, 2018).

Salicylic acid (SA), a natural plant hormone, act as an important signalling molecule triggering tolerance against abiotic and biotic stresses

(Hernández-Ruiz and Arnao, 2018; Gorni *et al*., 2020; Gorni *et al*., 2021). SA plays a significant role in many physiological and biochemical processes of the plant, being able to act as growth regulators and also as an abiotic elicitor capable of increasing the synthesis of secondary compounds beneficial to human health (Javanmardi and Akbari, 2016).

Ferulic acid (FA) is a phenolic compound synthetized from the metabolism of phenylalanine and tyrosine in plants. It is an important biological and structural component of the plant cell wall and it accumulates in soil and influences plant growth (Li *et al.*, 2013; Paiva *et al.*, 2013). FA is considered as non-enzymatic antioxidant and acts under stress to eliminate free radicals produced in plants (Andreasen *et al.*, 2001; Engwa, 2018). Studies on the exogenous application of FA in plants have demonstrated that this compound acts as an important regulator of several physiological processes related to plant growth and mitigating stress, such as, stomatal closure, cell division, membrane permeability, photosynthesis, respiration and many other metabolic processes (Santos *et al.*, 2008; Li *et al.*, 2013; Singh and Deen, 2014; Hussain *et al.*, 2017; Cheng *et al.*, 2018).

However, exogenous SA (Kazemi, 2014) and FA (Singh and Deen, 2014) applications were effective in inducing the growth and formation of secondary metabolites in tomato plants. Therefore, it is suggested that the application of SA and FA may result in combined effects of growth promotion and induction of biosynthetic pathways of secondary metabolism. Previous studies have revealed the hormonal and eliciting action of SA and FA (1.0 mmol L^{-1}) in tomato plants (Hussain *et al*., 2017; Kumar *et al*., 2017). In this context, the application of SA and FA can improve the productive performance and the biosynthesis of secondary compounds in tomato, making possible an increase in the commercial value of this crop, reaching greater market competitiveness. In this study we evaluated the effect of leaf spray of SA and FA either alone and in combination on the growth, yield and secondary compounds in tomato cultivar Santa Clara.

2. Materials and Methods

The experiment was conducted under greenhouse (without temperature and humidity control) covered with a 50% solar radiation shade located in Gammon Colleges, Paraguaçu Paulista (22°41'76" S, 50°58'33" W, 517 a.s.l.), Sao Paulo, Brazil.

Trademark tomato cultivar Santa Clara seeds were placed in a germination tray and after the training period (30 days) they were planted in 18 L pots containing soil. Soil samples were collected and submitted to chemical analysis according to Van Raij *et al.* (2001) (Table 1) and was corrected by applying limestone to increase the saturation of bases to 80% (40.0 g $pots^{-1}$), potassium chloride (3.0 g $pots^{-1}$) and simple super phosphate (20.0 g $pots^{-1}$). For fertilization with micronutrients it was added Yoorin Master 1 Si® (granulated) (Si: 10%, B: 0.1%, Mn: 0.3%, Cu: 0.05% and Zn: 0.55%) (1.5 g $pots^{-1}$), according to the recommendations of Bulletin 100 (IAC) for tomato species. The pots were irrigated by sprinklers per day at 8 a.m. and 5 p.m. in order to keep the soil moisture and ensure the availability of water throughout the experimental period.

The application of salicylic acid (SA: 138.121 g mol^{-1}) and ferulic acid (FA: 194.18 g mol^{-1}) was made for 3 consecutive days after having reached 20 days from transplant. Foliar applications of SA and FA at a dose of 1.0 mmol L^{-1} alone and in combination, and water-treated plants were used as control. SA and FA treatments were carried out by spraying the shoots of the plants with water-based solutions supplemented with Agral® (50 µL L^{-1} of solution) until the drop point (10 mL per plant), as follows: T1 - plants sprayed only with water (Control); T2 - applications of 1.0 mmol L^{-1} SA (SA); T3 - applications of 1.0 mmol L^{-1} FA (FA); and T4 - applications of 1.0 mmol L^{-1} SA + 1.0 mmol L^{-1} FA (SA + FA) by spraying the plants for three consecutive days.

Plants were harvested 90 days after transplanting the seedlings by collecting leaves, roots and fruits.

Plant growth

The effect of SA and FA on the plants were evalu-

Table 1 - Chemical analysis of the soil used in the experiment

pH	Organic matter g dm^{-3}	H + Al mmolc dm^{-3}	Ca mmolc dm^{-3}	Mg mmolc dm^{-3}	K mmolc dm^{-3}	P mg dm^{-3}
4.4	6.0	20.0	7.0	2.0	0.8	6.0

ated based on the fallowing: leaf area (cm^2), number of leaves per plant, number of fruits per plant, plant height (cm), dry weight of shoot and roots (g $plant^{-1}$) and mean fruit weight (g $plant^{-1}$). The leaf area was assessed using ImageJ® Software (Powerful Image Analysis) with 5 plants per treatment. The number of leaves and fruit per plant was determined by manual counting, considering fully expanded leaves and fruits at harvest point. The plant height was determined by measuring tape. The shoot and root dry weight were determined after drying in an oven with air circulation at 70°C until constant weight.

Chlorophyll content

Chlorophyll content (µg mL^{-1}) was determined spectrophotometrically following extraction in acetone, according to the method of Lichtenthaler (1987). Fresh leaf tissue (0.6 g) was added in 7 mL of 80% acetone, the tubes were shaken and left to stand for 30 minutes. The readings were performed in a spectrophotometer at λ 663 and λ 645 nm.

Total soluble sugar content

Total soluble sugar content in fruit was determined according to the method described by Dubois *et al*. (1956) using glucose as the standard. Fresh tissue (0.1 g) was added in 10 mL of 80% ethanol, and macerated in a mortar and left to rest for 30 min. The analyzes followed with the addition of phenol (5%) and sulfuric acid (98 N). The readings were performed in a spectrophotometer at λ 490 nm.

Preparation of fruit tomato extract

Determinations of total polyphenolic compounds and total flavonoid were obtained from the tomato juice. The fruits were ground in a low speed food processor (3000 rpm) for two minutes and passed in 2 mm sieves.

Total polyphenols contents

Total polyphenols contents were determined spectrophotometrically (λ 765 nm) by Folin-Ciocalteu method with modification described by Stagos *et al.* (2012). The total polyphenols contents were reported based on micrograms of gallic acid equivalents per milliliters (µg GAE mL^{-1}). Gallic acid solutions were prepared with concentrations of 25 to 500 µg mL^{-1} in absolute ethyl alcohol. The assays were performed in triplicate.

Total flavonoid content

Total flavonoid content was determined spectrophotometrically (λ 510 nm) by the method of Yao *et al.* (2013). The total flavonoids contents were reported based on micrograms of rutin equivalents per milliliters (µg RE mL^{-1}). Rutin solutions were prepared with concentrations of 25 to 500 µg mL^{-1} in absolute ethyl alcohol. The assays were performed in triplicate.

Carotenoid content

Carotenoid content were extracted from fresh tomato samples after homogenisation of whole fruits. The homogenised sample was mixtured with chloroform/acetone/ethanol (2:1:1, v/v/v) (Sadler *et al.*, 1990). For the determination of lycopene and β-carotene, the absorbance was read at λ 470 and λ 450 nm. Carotenoid content were determined according to the equation described by Craft and Soares (1992), using the molar extinction coefficient of 3450 for lycopene, and 2592 for β-carotene and expressed in micrograms per grams (µg g^{-1}). The assays were performed in triplicate.

Statistical analysis

The experiment was arranged in a completely randomized design with four treatments and five replications per treatment. The Shapiro-Wilk test was used to ensure the normality assumption and the homogeneity of variances. The data were submitted to analysis of variance ($p \leq 0.05$) and Tukey test ($p \leq 0.01$) using the SISVAR software. The results were presented by means of the treatments and standard error.

3. Results

The results indicated that SA and FA both caused a significant increase of growth in tomato (Table 2). The exogenous application of 1.0 mmol L^{-1} SA resulted in increasing in the accumulation of root dry weight by 61% and total dry weight by 41.3% compared to control plants. The application of 1.0 mmol L^{-1} FA resulted in increasing in shoot dry mass by 52.3% and total dry mass by 52% compared to control plants. However, the interaction between SA + FA resulted in increasing in shoot dry mass by 51.5%, root dry mass by 146.37% and total dry mass by 66.6%, when compared to control plants. Fruit weight increased by 16.5% in plant treated with 1.0 mmol L^{-1} FA.

The exogenous application of SA + FA resulted in increasing the leaf area by 47% and number of leaves by 204.3% compared to control plants (Table 3). Application of SA and FA showed increasing of 101.8

Table 2 - Effect of salicylic acid and ferulic acid on the shoot, root, total dry weight and fruit weight in tomato

Treatments	Shoot dry weight (g plant^{-1})	Root dry weight (g plant^{-1})	Total dry weight (g plant^{-1})	Fruit weight (g plant^{-1})
Control	24.01±1.11 c	4.55±0.83 c	28.57±1.60 c	524.46±2.07 b
SA	33.06±1.59 abc	7.32±0.36 b	40.38±1.60 b	554.29±7.44 b
FA	36.57±2.35 a**	6.84±0.42 bc	43.41±1.86 ab	610.78±11.94 a**
SA + FA	36.38±3.99 a	14.02±1.72 a**	47.59±4.57 a**	525.40±20.23 b
CV (%)	15.78	15.08	12.04	5.39

Different letters indicate significant differences by Tukey's test (p≤0.01).

Table 3 - Effect of salicylic acid and ferulic acid on leaf area, number of leaves per plant, number of fruits per plant and plant height in tomato

Treatments	Leaf area (cm²)	Number of leaves	Number of fruits	Plant height (cm)
Control	7955.4±466.9 b	65.2±3.69 c	20.8±0.86 b	77.4±2.67 ns
SA	8739.6±1054.1 ab	131.6±16.02 b	35.4±3.81 a **	79.6±2.75
FA	10209.8±850.6 ab	174.4±10.49 ab	35.0±3.20 a	88.2±7.20
SA + FA	11691.6±577.1 a *	198.4±10.09 a **	28.6±0.40 ab	77.0±1.04
CV (%)	16.11	16.71	19.89	10.75

Different letters indicate significant differences by Tukey's test (p≤0.01).

and 167.5% for the number of leaves, 70.2 and 68.3% for the number of fruits, when compared with control plants (Table 3). The plant height treated with SA and FA was not changed in relation to control plants.

Chlorophyll *a* content (Table 4) observed in tomato plants treated with SA, FA and the interaction with SA + FA were significantly higher by 26, 12 and 27.6%, respectively compared to control. Chlorophyll *b* and total chlorophyll decreased with the application the elicitors (Table 4). The total soluble sugar content in fruits, increased up to 36% in 1.0 mmol L^{-1} SA and 163% in 1.0 mmol L^{-1} FA treated plants when compared to the control.

The exogenous application of SA, FA and the interaction with SA + FA resulted in increasing in the accumulation of total polyphenols of 20.4, 110.3 and 12%, in relation to control plants (Table 5). Total flavonoid content in tomato were 81, 107 and 37% higher in SA, FA and the combined with SA + FA treatments when compared to control plants, respectively. In relation to carotenoid content plants treated with SA also presented increases of 157.3% (lycopene), and 157.7% (β-carotene). The combination of SA+FA showed increases of 65.25% (lycopene), and 65.3% (β-carotene), respectively, in comparison to the control (Table 5).

Table 4 - Effect of salicylic acid and ferulic acid on the chlorophyll and fruit total soluble sugar contents in tomato

Treatments	Chlorophyll *a* (μg mL^{-1})	Chlorophyll *b* (μg mL^{-1})	Total chlorophyll (μg mL^{-1})	Fruit total soluble sugar (mg g^{-1})
Control	9.66±0.23 c	21.02±0.13 a **	30.68±0.14 a **	22.63±0.96 c
SA	12.13±0.07 a	15.69±0.02 c	27.88±0.04 c	30.77±1.38 b
FA	10.82±0.04 b	13.03±0.08 d	23.90±0.05 d	59.47±1.92 a **
SA + FA	12.33±0.07 a **	16.05±0.02 b	28.45±0.04 b	26.89±0.21 bc
CV (%)	1.73	0.73	0.59	7.30

Different letters indicate significant differences by Tukey's test (p≤0.01).

Table 5 - Pair-wise genetic distance estimates based on observed phenotypes of 21 *Amaranthus* accessions

Treatments	Polyphenols (μg GAE mL^{-1})	Flavonoid (μg RE mL^{-1})	Lycopene (μg g^{-1})	β-carotene (μg g^{-1})
Control	245.58±7.21 c	272.33±4.81 d	7.54±0.16 c	10.03±0.2 c
SA	295.58±12.02 b	493.16±2.40 b	19.4±0.16 a**	25.85±0.2 a**
FA	516.42±0.00 a**	564.00±4.81 a**	6.96±0.16 c	9.25±0.2 c
SA + FA	274.75±4.81 b	372.33±4.81 c	12.46±0.00 b	16.58±0.0 b
CV (%)	2.60	1.85	2.39	2.17

Different letters indicate significant differences by Tukey's test (p≤0.01).

4. Discussion and Conclusions

Tomato plants treated with SA and FA presented higher total biomass (Table 2). The effect of elicitors plays a key role in growth regulation, plant development and these findings can be associated with the changes in hormone functions or the improvement of photosynthesis and carbohydrate accumulation in plants (Hussain *et al.*, 2017; Gorni *et al.*, 2020). The foliar application of SA and FA trigger a greater cellular activity that result in a larger number of leaves and leaf area, thus presenting a larger photosynthetic surface, improving the physiological processes (Gorni and Pahceco, 2016; Hussain *et al.*, 2017). Regarding the increase of leaf area, number of leaves, number of fruits and fruit weight, our results showed that the application of SA and FA can alter the growing pattern, in tomato plants (Table 3). The application of SA and FA increased the growth of leaves and roots, without changing the plant height (Khan *et al.*, 2003; de Carvalho *et al.*, 2020). Biomass gains as an effect of SA application were also observed in *Achillea millefolium* (Gorni and Pacheco, 2016), *Foeniculum vulgare* (Gorni *et al.*, 2017) and *Mentha spicata* (Kundu *et al.*, 2018). Increase in biomass, were also observed after FA application in plants of *Arabidopsis thaliana* (Reigosa and Pazos-Malvido, 2007), *Pisum sativum* (Orcaray *et al.*, 2011) and *Cucumis sativus* (Li *et al.*, 2013).

Pigments are the essential components for growth and development and they can determine the health status of plants (Hussain *et al.*, 2017; Gorni *et al.,* 2020). Based on our results, the application the elicitors SA and FA alone or in combination suggest an increase in chlorophyll *a* (Table 4). On the other hand, the application of SA and FA did not affect the chlorophyll *b* and total chlorophyll content, which directly reflected in the plant height values (Table 3). Anway, other studies conducted on different species reported increases in chlorophyll *a*, chlorophyll *b* and total chlorophyll contents consequences of SA treatment (Kazemi, 2014; Chakraborty *et al*., 2016) and in plants sprayed with FA (Zhu and Wakisaka, 2018). Positive effects of SA and FA on the physiological processes of tomato suggest that these elicitors may be associated with the regulation of several essential primary metabolic processes, including synthesis of chlorophylls (Zhu and Wakisaka, 2018; Gorni *et al*., 2020).

The positive effects of the application of SA and FA on the physiological processes of tomato plants were also reflected in higher soluble sugar content (Table 4). Sugar is one of the ingredients in tomato (Hafeznia *et al.*, 2014), and it is an important organic solute with low molecular weight in higher plants. The sugars which are accumulated under stress conditions or as consequence of treatments with SA or FA, keeping osmotic regulation and turgor inside the plant (Orcaray *et al.*, 2011; Gorni *et al.,* 2020). Results show that application of SA also resulted in increasing the total soluble sugar content in tomato plants (Kazemi, 2014), and chamomile (Zarinkamar *et al.*, 2013); similarly to application of FA in pea (Orcaray *et al.*, 2011) and soybean (Ferrarese *et al.*, 2001).

Polyphenols and flavonoids provide many physiological functions for plant survival and are of fundamental importance for plants adaptation (Verma and Shukla, 2015; Mehta *et al.*, 2018). Our results suggest that the application of SA and FA may modulate and alter the concentration of polyphenolic compounds (Table 5) in tomato plants. Studies report that the application of SA and FA stimulates the phenylpropanoid pathway, increasing the accumulation of polyphenols and flavonoids in plants (Salvador *et al.*, 2013; Gorni *et al.*, 2021).

Tomato plants have a good free radical scavenging capacity due to the high concentration of lyco-

pene and β-carotene. This pigment, it is the most effective antioxidant which gives color to tomato fruit as maturity progresses (Kumar *et al.*, 2017). Polyphenolic compounds and carotenoid act as antioxidants because they remove singlet oxygen and other free radicals in cells (Shahidi and Ambigaipalan, 2015) due to their ability to donate hydrogen from hydroxyl groups positioned along the aromatic ring to prevent oxidation by radicals free from lipids and other biomolecules (Sousa *et al.*, 2007).

Researchers showed an increase in carotenoid content during ripening due to the gradual degradation of chlorophyll (Adalid *et al.*, 2010). Our results showed that the application of SA and FA may modulate and alter the concentration of lycopene and β-carotene (Table 5) and chlorophyll *a* contents (Table 4) in tomato plants. Thus, our study evidenced that foliar application of SA and FA in tomato crop could enhance its antioxidant activity due to the higher concentration of these bioactive compounds. Our results corroborate with those found by Kumar *et al.* (2017), who also reported that foliar application of SA significantly increased lycopene content of tomato fruits. Singh *et al.* (2010) reported that application of L-phenylalanine and FA increased total polyphenols in pea plants.

Therefore, it can be concluded that application of bioregulators results in higher yield and higher concentration of secondary compounds of *Solanum lycopersicum*.

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