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Yield parameters and antioxidant compounds of tomato fruit: the role of plant defence inducers with or without *Cucumber mosaic virus* infection

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Abstract

BACKGROUND: The production of fruit and vegetables rich in health-promoting components in an eco-friendly context represents the winning answer to the world population demand for food. In this study, the effects of different treatments on the yield and fruit chemical characteristics of tomato (*Solanum lycopersicum* L.) are reported. The treatments included three inducers of plant defence responses (chitosan, *Trichoderma harzianum* T-22 and *Bacillus subtilis* QST713) applied alone or before *Cucumber mosaic virus* infection. Fruit production and antioxidant compounds were investigated by ultrahigh-performance liquid chromatography (UHPLC) and liquid chromatography–tandem mass spectrometry (LC–MS/MS).

RESULTS: Compared to control fruit harvested from untreated and healthy plants, treatment with QST713 increased the fruit number. Furthermore, plant treatments with T22, QST713 and chitosan alone enhanced fruit carotenoids (lutein and β-carotene), ascorbic acid and phenolic acids (caffeoyl glucoside and *p*-coumaroyl glucoside). In parallel, compared to fruit harvested from only CMV-infected plants, treatments with T22, QST713 and chitosan before CMV enhanced fruit ascorbic acid and flavonoids (quercetin 3-*O*-xylosyl-rutinoside and rutin).

CONCLUSION: Antioxidant compounds of tomato fruit can increase with the application of the plant defence inducers, thus protecting both the consumer and plant health.

Supporting information may be found in the online version of this article.

Keywords: *Cucumber mosaic virus*; defence inducers; fruit antioxidants; tomato; yield

INTRODUCTION

Increasing fruit and vegetable production represents a challenge to satisfy the world population food requirement. Furthermore, many people do not eat adequate quantities of fruit and vegetables.[1] Such crops should have excellent features, not only quantitatively but also qualitatively, reinforcing the human body against nutrition-related disorders and diseases.

The Mediterranean climate is well suited to tomato cultivation. Tomatoes are appreciated for their flavor and nutritional value, and consumed as a fresh or processed product (e.g. ketchup, juice, sauces, paste, puree).[2] Furthermore, tomato fruit can be regarded as a functional food[3] because of its content of antioxidant substances, such as ascorbic acid, lycopene and phenols,[4] thus playing a protective role in humans against cancer as well as chronic diseases.[5–7]

Indeed, antioxidants regulate levels of reactive oxygen species (ROS) such as superoxide, hydroxyl radical, hydrogen peroxide and singlet oxygen, which can cause cellular damage involving DNA, proteins and lipids.[8] In turn, the plant equilibrium between ROS generation and scavenging is perturbed by stress factors, such as pathogen infection, heavy metals, drought and high irradiance.[8]

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As a harmful plant pathogen, *Cucumber mosaic virus* (CMV) is able to cause the necrosis of tomato fruit and reduction in yield,[9] thus provoking economic damage. For this reason, several approaches, such as plant treatment with chitosan (CHT) and microorganisms, can represent an engaging strategy to manage CMV. Chitosan is a polycationic chitin derivative molecule, used for its plant defence-inducing, antimicrobial and film-forming properties.[10] Zhang *et al.*[11] reported that CHT inhibited grey mould (*Botrytis cinerea*) of cherry tomato fruit. In another study, Zhang *et al.*[12] observed that CHT treatment of fresh tomato fruit increased total phenolic and flavonoid content. In addition, microorganisms as biocontrol agents (BCAs) are able to control plant pathogens (both directly and indirectly by inducing plant defence responses), as well as to improve plant development and growth.[13] Among BCAs are recognized strains of the fungal genus *Trichoderma*[13] and the bacterial genus *Bacillus*. The last can also be classified as plant growth-promoting rhizobacteria (PGPR), acting directly, i.e. through the mechanisms of nitrogen fixation, phosphate solubilization, siderophore and phytohormone production.[14] Fousia *et al.*[15] demonstrated that *Bacillus subtilis* QST713 induced tomato plant defence against *Pseudomonas syringae* pv. *tomato* and increased the plant height.

Considering that CMV strain Fny, as well as CHT, *Trichoderma harzianum* strain T-22 (T22) and QST713, can change the plant physiology and metabolism, the focus of this study was to evaluate their effect on the yield and antioxidant content of fruit, in view of increasing the nutraceutical value of tomatoes.

MATERIALS AND METHODS

Sources and preparations of CMV and inducers

Tobacco (*Nicotiana tabacum* L. cv Xanthi) plants were adopted to propagate the necrosis-causing CMV strain Fny, obtained as indicated by Vitti *et al.*[16] Tobacco leaves showing symptoms of CMV-Fny were macerated in sodium citrate buffer (0.05 mol L^{-1}, pH 6.5) and, mechanically, the suspension was rubbed on the pre-dusted with celite tomato leaves. Low-molecular-weight CHT (50–190 kDa and 75–85% deacetylated) was obtained from Sigma-Aldrich (448 869; St Louis, MO, USA). 1 g CHT powder was dissolved in 40 mL distilled water containing 9 mL of 1 mol L^{-1} acetic acid. The pH value was 5.4. Inducing CHT solution was obtained by dissolving such stock (1 g) in distilled water (1 L), and spraying on the leaves (10 mL per plant). *Trichoderma harzianum* strain T22 (T22) as Trianum P was obtained from Koppert (Berkel en Rodenrijs, Netherlands), while *Bacillus subtilis* strain QST713 (QST713) as Serenade Max was obtained from Bayer CropScience (Leverkusen, Germany). According to the companies' instructions, the solutions of T22 (1.5 g 3.75 L^{-1} m^{-2}) and QST713 (3.25 kg 750 L^{-1} ha^{-1}) were prepared and used to drench the root zone (10^5 and 10^8 cfu per plant, respectively).

Experimental setup

Seeds of *Solanum lycopersicum* var. *cerasiforme* were sterilized by maintaining them for 1 min in a solution of 1% sodium hypochlorite. The seeds were then placed on sterile distilled water-imbibed moist filter paper in sterile Petri dishes to germinate. Seedlings grown after incubation, at 4 °C for 24 h in the dark and at 26 °C for 2–3 days, were planted in sterilized soil-filled pots. The soil was a mixture of loam, peat and sand in the proportion 1:1:1. Plants at the four-leaf stage were transplanted and grown in a greenhouse (26/23 °C day/night, 16 h photoperiod).

Tomato fruits were collected from plants, divided and treated following nine experimental conditions (15 plants per condition): fruit from control (untreated and healthy) plants (Control-PF); fruit from plants treated with T22 (T22-TPF); fruit from plants treated with QST713 (QST713-TPF); fruit from plants treated with CHT (CHT-TPF); fruit from plants infected with CMV (CMV-TPF); fruit from plants treated with T22 and CMV-infected after 7 days from T22 treatment (T22-CMV-TPF); fruit from plants treated with QST713 and CMV-infected after 7 days from QST713 treatment (QST713-CMV-TPF); fruit from plants treated with CHT and CMV-infected after 24 h from CHT treatment (CHT-CMV-TPF); fruit from plants treated with T22 and QST713 at the same time (T22 + QST713-TPF).

Treatments were performed from the tenth day after transplantation. Carotenoid, ascorbic acid and polyphenol (as well as total phenol) determinations were carried out in ripe tomato fruit, harvested 55 days after the (first) treatment on the plant.

Fruit weight and morphology

Ripe tomato fruits were harvested with 0.1 cm pedicel attached, starting from 55 days to 6 months after the (first) treatment on the plant. Fruit weight was determined using a digital scale (accuracy 0.01 g), while the measurements of the fruit polar and equatorial diameters were carried out using a Vernier caliper (accuracy 0.1 cm). The polar diameter was measured from the cutting point to the stigma position, while the equatorial diameter was measured considering the widest circumference of each fruit.

Carotenoid quantification

Sample preparation

Each sample (peel and pulp) was lyophilized for 24 h (LyoQuest-55, Telstar Technologies, Spain), reduced to a fine dried powder and kept at −20 °C until analysis. Dry sample (1 g), containing 0.1% butylated hydroxytoluene (BHT) as an antioxidant, was sonicated for 5 min with 10 mL hexane–ethyl acetate (1:1, v/v) and centrifuged at 19 000 × *g* for 15 min at 25 °C. The supernatant was removed and the solid residue was re-extracted with fresh extraction solvent, applying the same conditions. The whole procedure was repeated three times.[17]

Chromatographic conditions

Analyses using ultrahigh-performance liquid chromatography (UHPLC) were performed on a Nexera UHPLC system (Shimadzu, Kyoto, Japan); the quali-quantitative profile of carotenoids was performed according to Sommella *et al.*,[18] with few modifications. For the quantitative analysis of carotenoids, a Kinetex® EVO C18 column (150 × 2.1 mm, 100 Å) packed with 2.6 µm particles (Phenomenex, Bologna, Italy), was employed. The optimal mobile phase consisted of H_2O (A) and CH_3OH:ACN, 30:70 v/v (B). Analysis was performed in gradient elution as follows: 0–15.00 min, 60–95% B; 15–27.00 min, 95–97% B; 27–30.00 min, 97–100% B; 30–32.00 min, isocratic to 100% B; then 8 min for column re-equilibration. The flow rate was 0.5 mL min^{-1}. Column oven temperature was set to 40 °C. Injection volume was 3 µL extract. Chromatograms were monitored at 450 nm.

For their quantification, stock solutions were prepared in $CHCl_3$–CH_3OH (1:1, v/v); calibration curves were obtained in a concentration range of 0.5–300 µg mL^{-1} with six concentration levels, and triplicate injection of each level was run. Limits of detection (LOD) and quantification (LOQ) were calculated from the ratio between the standard deviation (SD) and

analytical curve slope multiplied by 3.3 and 10, respectively. For lutein: $y = 0.0001x - 0.4694$, linear correlation coefficient (R^2) = 0.9993, LOD = 0.211 μg mL^{-1}, LOQ = 0.638 μg mL^{-1}; for lycopene: $y = 0.0001x - 4.8689$, $R^2 = 0.9991$, LOD = 0.654 μg mL^{-1}, LOQ = 1.981 μg mL^{-1}; for β-carotene: $y = 0.0001x - 0.4081$, $R^2 = 0.9991$, LOD = 0.344 μg mL^{-1}, LOQ = 1.042 μg mL^{-1}. The amount of compounds in the fruit was expressed as milligrams per kilogram of dried extract.

Ascorbic acid quantification

Sample preparation

Dry sample (500 mg) was treated with 20 mL of 3% (w/v) metaphosphoric acid (HPO_3). The resulting solution was shaken for 30 min at 25 °C and centrifuged at 22 000 × *g* for 10 min. The supernatant was collected, filtered on 0.45 μm nylon membrane filters and injected into the HPLC system.[19]

Chromatographic conditions

UHPLC analyses were performed on a Shimadzu Nexera UHPLC system, using a 150 × 4.6 mm (2.7 μm) Ascentis® RP-Amide column (Supelco, Bellefonte, PA, USA) and setting the detector at 230 nm. Oven temperature was set to 35 °C. The mobile phase consisted of H_2O + 25 mmol L^{-1} NaH_2PO_4 (pH 3 with H_3PO_4) (A) and ACN (B). The flow rate was 0.8 mL min^{-1} and the injection volume was 3 μL sample. To determine the ascorbic acid content in tomato fruit, the analysis was performed in gradient elution: 0.01–4.00 min, isocratic to 2% B; 4.00–4.50 min, 2–95% B; 4.50–6.50 min, isocratic to 95% B; then isocratic to 2% B for column recondition and re-equilibration.

Instrumental calibration for the quantitative analysis was carried out using the external standard method, adopting the ascorbic acid as standard. The calibration curve was obtained in a concentration range of 10–200 μg mL^{-1} and triplicate injection was run ($y = 0.0001x + 0.5390$, $R^2 \geq 0.9983$). The amount of ascorbic acid was expressed as milligrams of vitamin C per gram of sample.

Total phenolic content

Total phenols were spectrophotometrically determined using the Folin–Ciocalteu method and catechol as standard. Each fruit (peel and pulp) was crushed and homogenized in 80% methanol +1% HCl solution by continuous stirring at 30 °C for 75 min. Homogenate was centrifuged for 15 min at 1000 × *g* and the supernatant was saved. 16 μL supernatant was diluted to 320 μL with water. 60 μL Folin–Ciocalteu reagent was added and the solution was left for 3 min. 200 μL of a 20% sodium carbonate solution was added and the resulting solution was mixed. Absorbance was measured at 650 nm after a dark incubation for 1 h.

Polyphenolic compound quantification

Sample preparation

Dry sample (1 g) was sonicated for 15 min with 10 mL methanol–water (80:20, v/v) at room temperature, and centrifuged at 19 000 × *g* for 20 min at 25 °C. The supernatant was collected and the extraction procedure was repeated three times for the complete recovery of polyphenolic compounds. The supernatants were combined, filtered on 0.45 μm filters (Phenex®-RC, Phenomenex) and injected.[20]

Identification of polyphenols by liquid chromatography–tandem mass spectrometry (LC-MS/MS)

LC-MS/MS analyses were performed on a Shimadzu Nexera UHPLC system coupled online to a liquid chromatography–mass spectrometry ion trap time-of-flight instrument through an electrospray ionization (ESI) source. The identification of polyphenolic compounds was performed according to Sofo *et al.*,[21] with slight modifications. Mobile phases were (A) H_2O and (B) ACN, both acidified by acetic acid (0.1%, v/v). Analysis was performed as follows: 0–10.00 min, 5–30% B; 10–12.00 min, 30–70% B; 12–13.00 min, 70–90% B; 13–14.00 min, isocratic to 90% B; 14–14.01 min, 90–5% B; 14.01–18.00 min, isocratic to 5% B. Flow rate was 0.4 mL min^{-1}. Column oven temperature was set to 45 °C. Injection volume was 2 μL. MS detection was operated in negative ESI mode with the following parameters: detector voltage, 1.55 kV; Curved Desolvation Line (CDL) temperature, 250 °C; block heater temperature, 280 °C; nebulizing gas flow (N_2), 1.5 L min^{-1}; drying gas pressure, 100 kPa. Full-scan MS data were acquired in the range 150–1200 *m/z* and MS/MS experiments were conducted in data-dependent acquisition; precursor ions were acquired in the range 170–800 *m/z*. Identification was carried out on the basis of standard retention time, UV spectra, comparing MS/MS data with those in the literature, and using Formula Predictor software (Shimadzu).

Quantification of polyphenolic compounds

Ultra-pressure liquid chromatography photodiode array (UPLC PDA) analyses were performed on a UPLC Acquity I-Class system (Milan, Italy) consisting of a Waters Acquity binary solvent manager, sample manager (FL), column manager (CM-A), PDA eLambda detector (equipped with a 500 nL detector flow cell volume), Acquity QDa detector and a degassing system. The whole configuration was driven by MassLynX v4.0 from Waters Corporation.

For the quantitative and qualitative analysis of polyphenolic compounds, a Kinetex® EVO C18 100 × 2.1 mm (100 Å) column packed with 1.7 μm particles (Phenomenex, Bologna, Italy) was employed. The optimal mobile phase consisted of 0.1% CH_3COOH–H_2O v/v (A) and 0.1% CH_3COOH–ACN v/v (B): 0–8.00 min, 2–30% B; 8–9.00 min, 30–95% B; 10–10.01 min, 95–2% B; then 3 min for column re-equilibration. The flow rate was 0.5 mL min^{-1}. Column oven temperature was set to 45 °C. Injection volume was 2 μL extract. The following PDA parameters were applied: sampling rate, 20 points s^{-1}; resolution, 1.2 nm. Data acquisition was set in the range 210–600 nm and chromatograms were monitored at 280 and 330 nm at the maximum absorbance of the compounds of interest.

For the quantification of polyphenol compounds, five compounds were selected as external standards (0.5–250 μg mL^{-1}): chlorogenic acid, kaempferol, naringenin, quercetin and rutin. A triplicate injection was run. The amount of compounds in the sample was expressed as milligram per gram of extract.

Statistical data analysis

Data normal distribution was tested using the Shapiro–Wilk test ($P \leq 0.05$) and homoscedasticity using Bartlett's test ($P \leq 0.05$). Data were analysed by one-way analysis of variance (ANOVA), parametric and non-parametric, performing Tukey's HSD and Kruskal–Wallis test, respectively. Statistical data analyses were carried out using RStudio software, version 1.0.136 (Integrated Development for R; Boston, MA, USA).

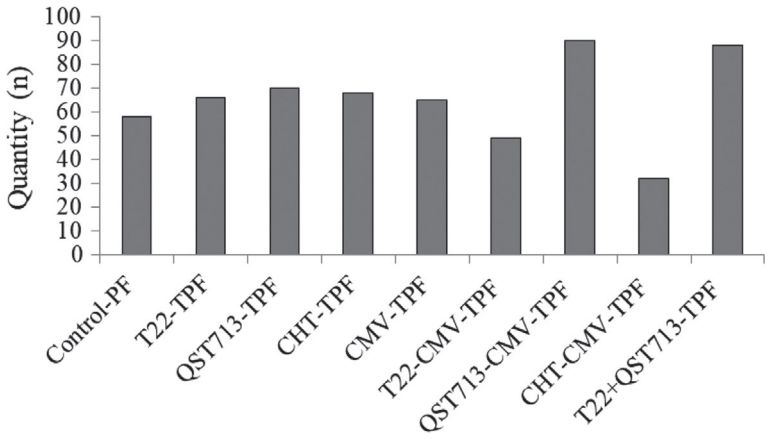

Figure 1. Number of ripe tomato fruit collected from 55 days to 6 months after the (first) plant treatment with inducers or CMV. Nine different experimental conditions were examined: fruit from control (untreated and healthy) plants (Control-PF); fruit from plants treated with T22 (T22-TPF); fruit from plants treated with QST713 (QST713-TPF); fruit from plants treated with CHT (CHT-TPF); fruit from plants infected with CMV (CMV-TPF); fruit from plants treated with T22 and CMV-infected after 7 days from T22 treatment (T22-CMV-TPF); fruit from plants treated with QST713 and CMV-infected after 7 days from QST713 treatment (QST713-CMV-TPF); fruit from plants treated with CHT and CMV-infected after 24 h from CHT treatment (CHT-CMV-TPF); fruit from plants treated with T22 and QST713 at the same time (T22 + QST713-TPF).

RESULTS AND DISCUSSION

Attention to the antioxidants in human health has motivated studies in horticulture and food science to evaluate fruit and vegetable antioxidants, and to assess how their characteristics can be influenced through cultivar development, production practices, storage and food processing.[22] This study reports the quantitative and qualitative effects on tomato fruit of plant treatments with the defence inducers CHT, T22, QST713 and the pathogen CMV.

Fruit production

Tomato fruit size, determined as weight or diameter, is conditioned by genetic and environmental factors.[23] Cell division and expansion processes determine cell number and size, respectively.[24] To increase yield, the positive effect of *Trichoderma viride* and *Pseudomonas fluorescens*, alone or combined, have been reported by Tanwar *et al.*[25] Our results showed that QST713-CMV-TPF and T22 + QST713-TPF were produced in the highest quantities (Fig. 1). This result is in agreement with Almaghrabi *et al.*,[26] who observed an enhanced number of tomato fruit per plant after treatment with *Bacillus* spp., *Pseudomonas* spp. and *Serratia marcescens*. Stimulation of marigold flower production by *Bacillus subtilis* BEB-13 has also been reported,[27] thus supporting the hypothesis that QST713 plays a role in the promoting plant flowering. Our results also showed that CHT-TPF was produced in greater quantity (17%), compared to control-PF (Fig. 1). This finding is in accordance with the increased number of flowers per plant and number of fruit per plant observed after CHT foliar application to tomato plants by Sathiyabama *et al.*[28]

The weight of CHT-CMV-TPF was significantly higher than that of all the other fruit (Fig. 2), although CHT-CMV-TPF was produced in the lowest quantity (Fig. 1). T22-CMV-TPF was the lightest (Fig. 2).

No significant difference was observed in the polar and equatorial diameters between control-PF, T22-TPF and T22 + QST713-TPF (Figs 3 and 4). Furthermore, no significant change in diameter was observed between QST713-TPF and control-PF (Figs 3 and 4), as obtained by Karakurt *et al.*,[29] who evaluated the *Bacillus subtilis* OSU-142 tree application effect on sour cherry. Hence our findings demonstrated that T22 and QST713 were ineffective as fruit size promoters, both alone and combined. CMV-TPF size was smaller than that of control-PF, even if not significantly (Figs 2–4). Interestingly, CHT-CMV-TPF and T22-CMV-TPF showed the largest and smallest size, respectively (Figs. 2–4).

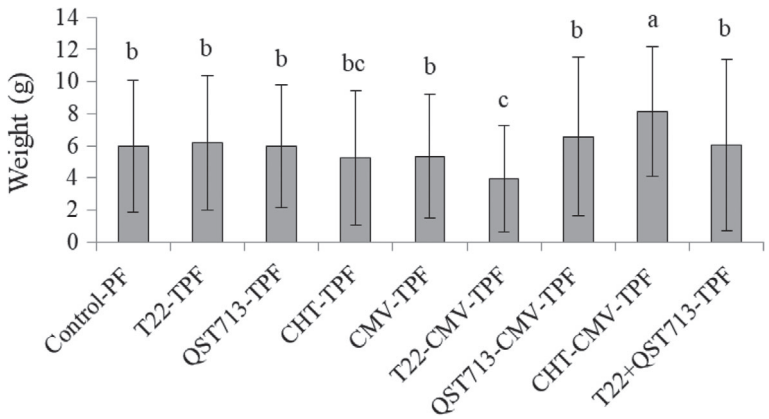

Figure 2. Weight of tomato fruit. Mean values ($32 \leq n \leq 90$) are represented. Standard deviations are represented by bars. Significant differences ($P \leq 0.05$) among treatments are indicated by different letters, according to non-parametric one-way ANOVA. Experimental conditions as Fig. 1.

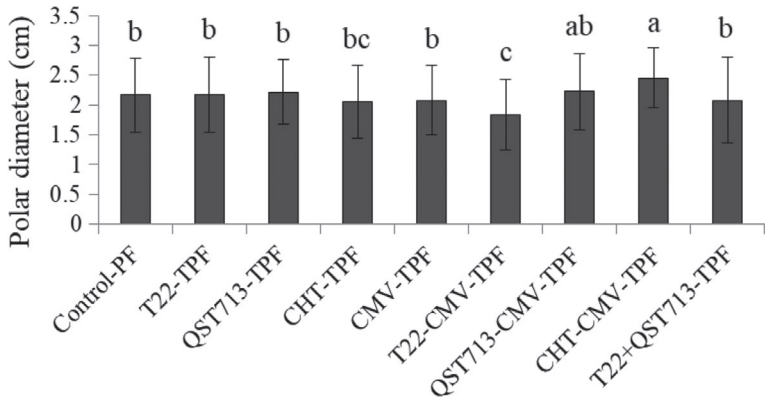

Figure 3. Polar diameter of tomato fruit. Mean values ($32 \leq n \leq 90$) are represented. Standard deviations are represented by bars. Significant differences ($P \leq 0.05$) among treatments are indicated by different letters, according to non-parametric one-way ANOVA. Experimental conditions as Fig. 1.

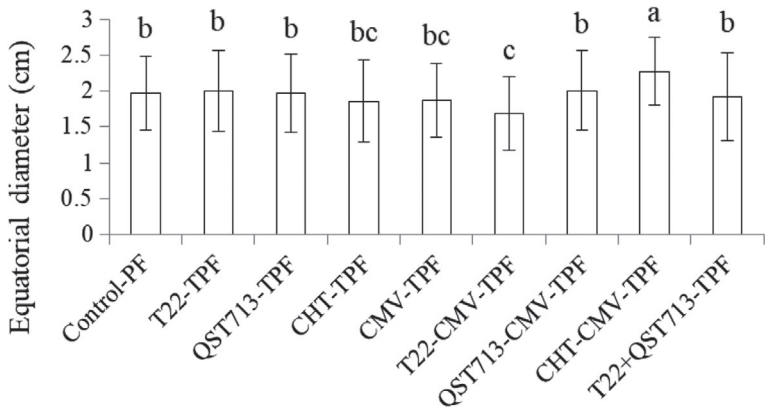

Figure 4. Equatorial diameter of tomato fruit. Mean values ($32 \leq n \leq 90$) are represented. Standard deviations are represented by bars. Significant differences ($P \leq 0.05$) among treatments are indicated by different letters, according to non-parametric one-way ANOVA. Experimental conditions as Fig. 1.

Fruit carotenoid (lutein, lycopene and β-carotene) content

Plant carotenoids, such as lutein, lycopene and β-carotene, are mainly inserted in chromoplasts and chloroplast membranes, where color of flowers/fruit also play a role in photoprotection and photosynthesis.[30] The decrease in chlorophylls and increase in lycopene are responsible for the color change from green to red, occurring during tomato fruit development.[31] Carotenoids cannot be synthesized by animals, and the interest in dietary carotenoids is due to their ability as moderators of chronic diseases.[5] The benefit of carotenoids in the human diet has prompted plant product engineering to improve carotenoid accumulation.[32] It is known that phytohormones (as auxin, ethylene and abscisic acid) participate in carotenoid accumulation and regulation of ripening of tomato fruit.[32] Furthermore, environmental factors (e.g. radiation intensity, temperature and CO_2 concentration) and genetic potential can change the metabolism of carotenoid in tomato fruit.[32] In cherry tomato fruit, D'Evoli *et al.*[33] found that lycopene concentration in raw pulp was about fourfold lower than in raw skin, and similar results were obtained for β-carotene, even if at a lower degree than lycopene. In this study, the lutein content was significantly higher in all fruit of the treated plants compared to control-PF. Specifically, T22-TPF showed the highest lutein content (Table 1). The most significant difference in lycopene amount was between T22-TPF as well as CHT-TPF, compared to QST713-TPF (Table 1). Recently, Osano *et al.*[34] also reported an increased lycopene content in tomato fruit of pre-harvest CHT-treated plants. Finally, β-carotene can be converted into vitamin A.[35] In our study, T22-TPF, QST713-TPF, CHT-TPF and CMV-TPF showed a significantly higher β-carotene value compared to control-PF (76%, 62%, 64% and 23%, respectively; Table 1). The significantly lowest β-carotene value was in CHT-CMV-TPF (Table 1).

Table 1. Lutein, lycopene and β-carotene content of tomato fruit. Mean values ($n = 4$) ± standard deviation are represented

Condition	Lutein (mg kg^{-1})	Lycopene (mg kg^{-1})	β-Carotene (mg kg^{-1})
Control-PF	20.10 ± 1.39e	359.63 ± 5.72e	93.55 ± 5.01e
T22-TPF	63.40 ± 1.51a	584.00 ± 18.92a	164.33 ± 3.30b
QST713-TPF	46.48 ± 2.25c	223.75 ± 14.71g	151.15 ± 2.44c
CHT-TPF	47.85 ± 2.41c	555.48 ± 18.43a	153.80 ± 7.26c
CMV-TPF	29.33 ± 0.34d	469.85 ± 19.59bc	114.83 ± 5.07d
T22-CMV-TPF	55.70 ± 0.42b	446.18 ± 9.75cd	199.88 ± 1.12a
QST713-CMV-TPF	61.15 ± 2.00a	506.95 ± 31.40b	146.35 ± 4.40c
CHT-CMV-TPF	27.98 ± 0.92d	419.73 ± 1.09d	11.53 ± 0.68f
T22 + QST713-TPF	52.80 ± 1.72b	303.20 ± 20.90f	113.53 ± 1.09d

Significant differences ($P \leq 0.05$) among treatments are indicated by different letters, according to parametric one-way ANOVA. Experimental conditions as in Fig. 1.

Table 2. Ascorbic acid content of tomato fruit. Mean values ($n = 4$) ± standard deviation are represented

Condition	Ascorbic acid (mg g^{-1})
Control-PF	2.19 ± 0.03i
T22-TPF	3.60 ± 0.02e
QST713-TPF	4.57 ± 0.05a
CHT-TPF	3.06 ± 0.06h
CMV-TPF	3.47 ± 0.03f
T22-CMV-TPF	3.97 ± 0.07c
QST713-CMV-TPF	4.39 ± 0.03b
CHT-CMV-TPF	3.80 ± 0.02d
T22 + QST713-TPF	3.29 ± 0.05g

Significant differences ($P \leq 0.05$) among treatments are indicated by different letters, according to parametric one-way ANOVA. Experimental conditions as in Fig. 1.

Fruit ascorbic acid (vitamin C) content

Absence of L-gulonolactone oxidase does not allow humans to synthesize ascorbic acid. Furthermore, it is water soluble and not stored in the human body.[36] Ascorbic acid is involved in several mechanisms, such as collagen maintenance, carnitine and catecholamine synthesis, cholesterol transformation, common cold prevention or relief and scurvy prevention.[36] Gahler *et al.*[37] reported that vitamin C was lost during production of tomato juice and baked tomatoes owing to its heat instability. The results demonstrate that the ascorbic acid content of T22-TPF and QST713-TPF were significantly higher than that of control-PF (64% and 109%, respectively) (Table 2). Interestingly, the ascorbic acid content of T22 + QST713-TPF was significantly higher than that of control-PF (50%) but lower than that of T22-TPF and QST713-TPF (−9% and − 28%, respectively) (Table 2). Also, the ascorbic acid content of CHT-TPF was significantly higher (40%) than that of control-PF (Table 2). A similar result was obtained by Almunqedhi *et al.*,[38] who used CHT spray applications during the pre-harvest phase. As a defence response to the virus, Fujiwara *et al.*[39] reported an accumulation of ascorbic acid in *Brassica rapa* plants resistant to *Turnip mosaic virus*. Finally, all the treatments provided fruit that was significantly higher in ascorbic acid than control-PF (Table 2).

Fruit total phenolic content

Plant phenolics are mainly involved in resistance to pathogens, growth, reproduction and pigmentation mechanisms.[40]

In our study, the total phenolic content of CHT-CMV-TPF was significantly higher (79%) than that of CHT-TPF, the highest and the lowest value respectively (Fig. 5). Total phenols of CHT-TPF were significantly lower than those of control-PF (Fig. 5). Differently, the CHT coating enhanced the total phenolic content of table grapes during storage for 60 days at 0 °C.[41] No significant difference in phenol amount was observed between T22-TPF, QST713-TPF, T22 + QST713-TPF and control-PF (Fig. 5). Differently from our results, Rahman *et al.*[42] observed that *Bacillus amyloliquefaciens* BChi1 and *Paraburkholderia fungorum* BRRh-4 significantly increased the total phenolic content in strawberry fruit.

Fruit polyphenolic compounds content

Plant polyphenols include phenolic acids, flavonoids, lignans and stilbenes, also associated with organic acids and carbohydrates.[43] The flavonoid family includes flavanonols, flavanols, isoflavones, flavonols, flavones, flavanones and anthocyanidins. Among the main dietary flavonols, there are kaempferol (found in tea and beans, for example) and quercetin (found in onions and chocolate, for example).[44] The results showed that fruit of treated plants had a caffeoyl glucoside content significantly higher than that of control-PF; in particular, T22 + QST713-TPF had the significantly highest content (Table 3). Quantities of *p*-coumaroyl glucoside detected in control-PF and CHT-CMV-TPF were significantly lower than those determined in fruit of the other treated plants, and particularly CHT-TPF had the significantly highest content (Table 3). Moghadam *et al.*[45] reported the wound healing potential of chlorogenic acid isolated from *Parrotia persica*. In our case, the content of 3-*O*-caffeoylquinic acid and 5-*O*-caffeoylquinic acid detected in T22-TPF was significantly higher than that detected in fruit of other plants; in addition, the significantly lowest quantity of caffeoylquinic acid (isomer) was detected in T22 + QST713-TPF (Table 3). The lowest and significantly highest content of quercetin 3-*O*-xylosyl-rutinoside were detected in control-PF and QST713-CMV-TPF, respectively (Table 3). The significantly highest rutin content was detected in all fruit harvested from the treated and then infected plants (referring to T22-CMV-TPF, QST713-CMV-TPF and CHT-CMV-TPF) (Table 3). Mikulic Petkovšek *et al.*[46] obtained higher rutin content in apple leaves infected by *Venturia inaequalis* compared to healthy leaves.

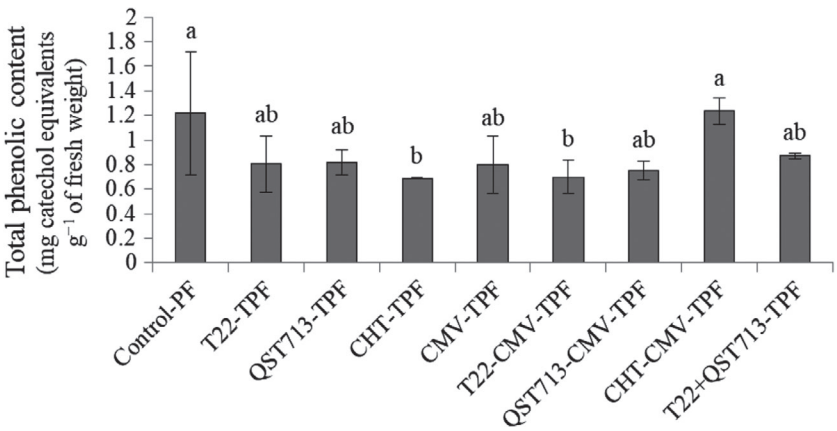

Figure 5. Total phenolic content of tomato fruit. Mean values ($n = 4$) are represented. Standard deviations are represented by bars. Significant differences ($P \leq 0.05$) among treatments are indicated by different letters, according to parametric one-way ANOVA. Experimental conditions as Fig. 1.

Table 3. Polyphenolic compound content of tomato fruit. Mean values ($n = 6$) ± standard deviation are represented

Condition	Polyphenols (mg g^{-1})										
	Caffeoyl glucoside	*p*-Coumaroyl glucoside	3-*O*-Caffeoylquinic acid	5-*O*-Caffeoylquinic acid	Caffeoylquinic acid (isomer)	Quercetin 3-*O*-xylosyl-rutinoside	Rutin	Kaempferol 3-*O*-rutinoside	Naringenin glucoside	Naringenin	Naringenin chalcone
Control-PF	0.071 ± 0.010h	0.056 ± 0.002e	0.635 ± 0.069cd	1.038 ± 0.135de	0.267 ± 0.038c	0.134 ± 0.013e	1.212 ± 0.161f	0.092 ± 0.013de	UnderLOQ	UnderLOQ	6.705 ± 0.636de
T22-TPF	0.134 ± 0.003d	0.075 ± 0.002d	0.870 ± 0.028a	1.558 ± 0.047a	0.242 ± 0.008cd	0.198 ± 0.005c	2.944 ± 0.157c	0.098 ± 0.004c	0.026 ± 0.021d	0.022 ± 0.021b	7.889 ± 0.671cd
QST713-TPF	0.135 ± 0.002d	0.098 ± 0.003c	0.714 ± 0.009b	1.213 ± 0.017bc	0.319 ± 0.003ab	0.248 ± 0.003b	2.809 ± 0.097c	0.113 ± 0.001ab	0.038 ± 0.001c	0.009 ± 0.004c	7.433 ± 0.430cd
CHT-TPF	0.223 ± 0.009b	0.155 ± 0.007a	0.309 ± 0.070e	0.620 ± 0.034f	0.265 ± 0.020c	0.145 ± 0.005e	1.096 ± 0.059f	0.095 ± 0.004cd	0.014 ± 0.004e	UnderLOQ	2.710 ± 0.248g
CMV-TPF	0.124 ± 0.002e	0.074 ± 0.001d	0.674 ± 0.008bc	1.133 ± 0.017cd	0.235 ± 0.003d	0.173 ± 0.002d	1.866 ± 0.048e	0.089 ± 0.003ef	0.024 ± 0.003d	0.028 ± 0.009ab	11.370 ± 1.134b
T22-CMV-TPF	0.149 ± 0.006c	0.123 ± 0.007b	0.598 ± 0.026d	0.980 ± 0.052e	0.343 ± 0.016a	0.255 ± 0.008b	3.681 ± 0.228b	0.134 ± 0.004a	0.106 ± 0.006a	UnderLOQ	4.961 ± 0.596f
QST713-CMV-TPF	0.113 ± 0.007f	0.095 ± 0.009c	0.726 ± 0.034b	1.247 ± 0.066bc	0.306 ± 0.017b	0.337 ± 0.014a	3.958 ± 0.129a	0.109 ± 0.003b	0.053 ± 0.007b	UnderLOQ	5.504 ± 0.531ef
CHT-CMV-TPF	0.087 ± 0.008g	0.061 ± 0.008e	0.729 ± 0.047b	1.277 ± 0.087b	0.244 ± 0.016cd	0.193 ± 0.010c	3.749 ± 0.095b	0.080 ± 0.003g	UnderLOQ	0.032 ± 0.011ab	15.004 ± 1.734a
T22 + QST713-TPF	0.347 ± 0.007a	0.113 ± 0.004b	0.336 ± 0.007e	0.502 ± 0.019f	0.188 ± 0.009e	0.192 ± 0.006c	2.037 ± 0.051d	0.085 ± 0.004fg	0.100 ± 0.009a	0.033 ± 0.003a	8.645 ± 0.323c

Significant differences ($P \leq 0.05$) among treatments are indicated by different letters, according to parametric and non-parametric one-way ANOVA. Experimental conditions as in Fig. 1.

Furthermore, we detected a significantly lower rutin content in T22 + QST713-TPF compared to T22-TPF and QST713-TPF (−31% and −27%, respectively; Table 3). Ggotek *et al.*[7] reported a wide scenario of rutin effects on UV-irradiated human skin fibroblasts, including apoptotic balance regulation as well as inflammation and redox imbalance prevention. In our study, the most significant difference in kaempferol 3-*O*-rutinoside content was between T22-CMV-TPF and CHT-CMV-TPF (Table 3). Petpiroon *et al.*[47] observed the promotion of keratinocyte migration by kaempferol-3-*O*-rutinoside, suggesting the involvement of this molecule in wound healing therapies. We obtained the two significantly highest naringenin glucoside contents in T22-CMV-TPF and T22 + QST713-TPF (Table 3). Furthermore, the highest naringenin content determined in T22 + QST713-TPF was significantly higher than that of T22-TPF and QST713-TPF (50% and 267%, respectively; Table 3). For the first time, Álvarez-Álvarez *et al.*[48] reported that naringenin is naturally produced also by *Streptomyces clavuligerus*. Naringenin and its glycoside naringin were assayed, evidencing that naringenin was more efficient as a hydroxyl/superoxide radical scavenger and demonstrated higher antioxidant capacity than naringin. However, both flavanones equally reduced DNA damage.[49] Yilma *et al.*[50] reported the ability of naringenin to modulate the inflammatory responses triggered by *Chlamydia trachomatis* in mouse macrophages. Chang *et al.*[6] reported the role of naringenin as an inhibitor of human lung cancer cell migration. Interestingly, in leaves of cucumber plants treated with *Trichoderma atroviride* TRS25, Nawrocka *et al.*[51] observed increased caffeoylquinic acid, rutin and naringenin quantities, likely contributing to plant protection. We detected the significantly highest and lowest content of naringenin chalcone in CHT-CMV-TPF and CHT-TPF, respectively (Table 3). Iwamura *et al.*[52] demonstrated that naringenin chalcone, purified from red tomato skin, suppressed allergic asthma symptoms exhibited in mice. Malhotra *et al.*[53] observed that flavonoids and related compounds reduced the infectivity of tomato ringspot virus in *Chenopodium quinoa*. This finding could explain the significant increase of quercetin 3-*O*-xylosyl-rutinoside and rutin in fruit harvested from treated then CMV-infected plants, compared to fruit from the treated-only plants (Table 3), considering the plant defence-inducing properties of T22, QST713 and CHT. Finally, a higher flavonoid content was detected in QST713-TPF compared to control-PF (Table 3). This finding is in accordance with Rahman *et al.*,[42] who also observed that plant treatments with *Bacillus amyloliquefaciens* BChi1 and *Paraburkholderia fungorum* BRRh-4 increased the flavonoid content of strawberry fruit.

Vitti *et al.*[16] observed that tomato plants infected with CMV exhibited a strong ROS augmentation. Although antioxidants are known for their ability to scavenge ROS,[8] unfortunately, to date there is a lack of scientific evidence characterizing a mechanism for the direct action of antioxidant compounds against viruses in plants; thus further studies are needed.

However, our findings may also apply to other crops extended to the national and international market and, overall, inducers can reduce risks to humans and environment.

Of note, Martí *et al.*[54] reviewed the involvement of tomato carotenoids and polyphenols in cancer prevention, and reported strategies to improve their content in fruit. Moreover, they also reported some studies revealing carotenoids associated with a higher cancer risk/incidence/mortality, manifested especially among smokers and diagnosed breast cancer. Finally, supporting our study, Fraser and Bramley[5] affirmed that carotenoids from fruit and vegetables, combined with other antioxidants,

are more efficient than dietary supplements of individual compounds.

CONCLUSIONS

This paper reports the effects of plant treatments with CMV and inducers of defence responses chitosan, *Trichoderma harzianum* T-22 and *Bacillus subtilis*.

Regarding QST713 on ripe tomato fruit, overall, QST713 can be considered as the most effective for increasing the fruit number as well as T22 and QST713 for increasing ascorbic acid and the majority of carotenoids and polyphenols assayed in fruit. Hence the use of inducers can be recommended to benefit consumer and plant health, in the context of sustainable agricultural practices.

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SUPPORTING INFORMATION

Supporting information may be found in the online version of this article.

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