# Foliar applications of a legume-derived protein hydrolysate elicit dose-dependent increases of growth, leaf mineral composition, yield and fruit quality in two greenhouse tomato cultivars

**Youssef Rouphael[a*], Giuseppe Colla[b], Maria Giordano[a], Christophe El-Nakhel[a], Marios C. Kyriacou[c], Stefania De Pascale[a]**

[a]*Department of Agricultural Sciences, University of Naples Federico II, 80055 Portici, Italy*

[b]*Department of Agricultural and Forestry Sciences, University of Tuscia, 01100 Viterbo, Italy*

[c]*Department of Vegetable Crops, Agricultural Research Institute, 1516 Nicosia, Cyprus*

*Corresponding author. Phone: +39 (081) 2539134; Fax: +39 (081) 7755129

*E-mail address*: youssef.rouphael@unina.it (Y. Rouphael)

**Abstract**

The use of natural plant biostimulants is proposed as a promising and innovative approach to ensure improved and sustainable yields and product quality. A greenhouse experiment was performed to assess the yield performance, leaf net assimilation of $CO_2$, mineral composition of leaves and fruits, and fruit physicochemical quality attributes of two tomato cultivars (Akyra and Sir Elyan) in relation to biostimulant treatments (control or two different concentrations of the legume-derived protein hydrolysate Trainer®). Treated tomato plants were sprayed every 10 days with a solution containing 2.5 and 5.0 ml $L^{-1}$ of biostimulant. Akyra was found to be richest in K, Ca, Mg, lipophilic and hydrophilic antioxidant activities (LAA and HAA), lycopene, total phenolic and total ascorbic acid. Foliar applications of legume-derived protein hydrolysate at 5.0 ml $L^{-1}$ increased marketable yield of Akyra and Sir Elyan by modulating yield components differently depending on cultivars: higher number of fruits in Akyra and increase fruit mean weight in Sir Elyan. Improved yield performance with biostimulant foliar applications at the highest rate was related to improved leaf nutritional status (higher K and Mg) and higher net assimilation of $CO_2$. The application of legume-derived protein hydrolysate at 5.0 ml $L^{-1}$, and to a lesser degree at 2.5 ml $L^{-1}$, elicited an increase in antioxidant activities, total soluble solids, mineral composition (K and Mg) as well as bioactive molecules such as lycopene and ascorbic acid, thereby increasing the nutritional and functional quality of the fruits. These findings can assist tomato growers in selecting cultivars and application dose for protein hydrolysate to complement high crop productivity with optimal fruit quality.

*Keywords:* Antioxidant activity, lycopene, mineral composition, plant biostimulants, *Solanum lycopersicum* L., sustainable horticulture.

## 1. Introduction

In the coming years, food demand will rise steadily with concomitant increase in the global population. Thus, a fundamental challenge for agriculture is to produce sufficient food and, at the same time, minimize collateral damage to the environment (Duhamel and Vandenkoornhuyse, 2013). In other words, new farming practices should be introduced in order to produce more food in a sustainable way *(i.e.*, by improving resource use efficiency). The use of natural plant biostimulants has been proposed as a promising, safe and meaningful approach to address the sustainability challenges facing horticulture and to ensure high yield and quality of horticultural commodities (Colla and Rouphael, 2015; du Jardin, 2015). As defined by the Association of American Plant Food Control Officials (www.aapfco.org) plant biostimulants are '*any substance or compound other than primary (i.e., N, P, K) secondary (i.e., Ca, Mg, S) and microplant nutrients (i.e., Fe, Mn, Zn, Cu), that can demonstrate by scientific research to be beneficial to one or more plant species when applied exogenously*'.

According to Colla et al. (2015) protein hydrolysates (PHs) represent a notorious category of plant biostimulants (PBs) defined as *'mixtures of amino acids, polypeptides and oligopeptides that are manufactured from animal or plant protein sources using partial hydrolysis'* (Schaafsma, 2009). They are available in the market as liquid products, soluble powder or granular form, and can be applied as seed treatment, foliar spray and soil drench (Colla et al., 2015). PHs coming from vegetal source of proteins are gaining interest worldwide because of their superior agronomic value compared to animal-derived PHs (Cerdán et al., 2009) and the lack of restrictions in the use of plant-derived PHs in organic farming.

Recent studies reported that foliar and/or root applications of PHs are capable of eliciting an array of physiological processes in crops that stimulate growth (Colla et al., 2014), enhance yield and product quality (Colla et al., 2017; Ertani et al., 2014), improve tolerance to a wide range of abiotic stresses, including drought (Feitosa de Vasconcelos et al., 2009), salinity (Ertani et al., 2013; Lucini et al., 2015), thermal (Botta, 2013) and nutrient stress (Colla et al., 2013) as well as adverse

soil pH (Rouphael et al., 2017a). Direct effects of PHs on plants include fostering the activity of key enzymes (*i.e.*, asparate aminotransferase, glutamate synthase, glutamine synthetase, nitrate reductase and nitrite reductase) involved in N reduction and assimilation as well as C metabolism (Colla et al., 2015; du Jardin, 2015; Schiavon et al., 2008). Moreover, PHs application could also elicite auxin- and gibberellin-like activities due to the presence of bioactive peptides, thus boosting crop performance (Colla et al., 2014; Ertani et al., 2009; Matsumiya and Kubo, 2011). In addition to direct effects, the application of PHs has been shown to modify root growth and architecture (*i.e.* indirect effects), facilitating the macro- and microelement uptake as a consequence of the increased root surface area (density, length and number of lateral roots; Colla et al., 2015; du Jardin, 2015; Ertani et al., 2009).

However, except from a few studies, the potential benefits from the application of plant-derived PHs on vegetable crops were mainly investigated at tissue level (*i.e.* bioassays) and at seedling stage under soilless and substrate culture (Colla et al., 2013, 2014; Lucini et al., 2015; Matsumiya and Kubo, 2011). Limited information is available concerning the effect of foliar applications of plant-derived PHs on agronomical, physiological and fruit quality responses of an important vegetable crop such as fresh-market tomato grown under soil conditions. Moreover, the biostimulant action of PHs can vary depending on species and/or cultivars, growing seasons, mode and dose of applications (Colla et al., 2015). Thus, understanding how the dose of applications and their interaction with cultivars can modulate crop productivity and physico-chemical attributes of greenhouse tomato under soil culture is an urgent need among vegetable farmers, extension specialists and researchers for the continued success of the use of natural biostimulants.

Accordingly, the objectives of the current study were: i) to examine the response of two greenhouse tomato cultivars to a legume-derived PH, delivered by foliar application at three concentrations, in terms of yield performance characteristics, leaf net $CO_2$ assimilation rate, mineral composition and fruit physicochemical composition, and ii) to assess the associations between these nutritive traits.

## 2. Materials and methods

### *2.1. Experimental conditions and tomato cultivars*

The experiment was carried out in the summer-autumn 2016, in an unheated greenhouse covered by ethyl vinyl acetate 0.2 mm plastic film, situated at Somma Vesuviana, Naples province, South Italy (lat. 40° 53' 14" N, long. 14° 28' 40" E; altitude 155 m above sea level). The mean air temperature and relative humidity inside the greenhouse were 22 ºC and 65%, respectively. The soil was sandy (85% sand, 7% silt, 8% clay), with a bulk density of 1.1 g $cm^{-3}$, pH of 7.5, electrical conductivity of 1.2 dS $m^{-1}$, organic matter of 0.8% (w/w), total N at 0.03%, available P at 16 mg $kg^{-1}$ and exchangeable K at 35 mg $kg^{-1}$.

Two tomato (*Solanum lycopersicum* L.) F1 hybrids were tested in the current greenhouse experiment, cvs. Sir Elyan (Vilmorin INC, Tucson, USA) and Akyra (Syngenta, Milan, Italy). The Sir Elyan cultivar is characterized by a medium-size plum-shape fruit (70-100 g), good potential for fruit setting and production and low sensitiveness to blossom end rot, whereas the mini plum Akyra cultivar has an average fruit weight between 30-40 g (small size) with 15 to 20 fruits per truss, and characterized by its high resistance against cracking and its long shelf life. Both cultivars are harvested at full ripening stage and are widely cultivated under protected cultivation in Italy. At the two true-leaf stage tomato seedlings were transplanted on 3 August 2016. Plant rows were 0.9 m apart, and the space between plants within a row was 0.3 m. The distance between the centers of double rows was 2.22 m, resulting in a plant density of 3.0 plants $m^{-2}$, as used commercially for fresh tomato under greenhouse conditions.

### *2.2. Experimental design, protein hydrolysate application and crop management*

Six treatments derived by the factorial combination of two indeterminately growing plum tomato cultivars (Sir Elyan and Akyra) and three biostimulant applications (control or two concentrations of plant-derived protein hydrolysate 2.5 or 5.0 ml $L^{-1}$). Treatments were arranged in

a randomized complete-block design with three replicates per treatment amounting to a total of 18 experimental unit plots. Each experimental unit consisted of a 10 $m^2$ plot area containing 30 plants ($n$ = 540 plants).

The commercial plant-derived PH biostimulant Trainer® (Italpollina S.p.a, Rivoli Veronese, Italy) obtained through enzymatic hydrolysis of proteins from legume seeds was used in the current experiment. Trainer® contains mostly soluble peptides (290 g $kg^{-1}$) and carbohydrates (90 g $kg^{-1}$) and to a lesser extent free amino acids (10 g $kg^{-1}$).

The treated plants were uniformly sprayed nine times during the growing cycle at 10-day intervals using a 30-L stainless steel sprayer (Taizhou Dili Trading Co., Ltd, Zhejiang, China). Foliar application was initiated 15 days after transplanting (17 August). The 2.5 ml $L^{-1}$ concentration of Trainer® was used based on the manufacturer's recommendations, whereas the 5.0 ml $L^{-1}$ dose was adopted in order to evaluate whether it could further enhance the tomato performance. Crop was pruned at the $6^{th}$ truss stage. Pollination was facilitated by bumble bees introducing a 'Natupol' hive (Koppert Italia S.R.L., Bussolengo, Italy) at full flowering of the first truss. During the growing season, pests (spider mites, leafminers, aphids) and pathogens (late blight, downy mildew, grey mould) were controlled based on standard commercial practices used in Italy.

*2.3. Nutrient solution management*

Fertilizer was applied through the drip irrigation system consisting of drip lines placed 5 cm away from the tomato plants, with in-line emitters located 0.30 m apart and an emitter flow of 2.4 L $h^{-1}$. The nutrient solution was delivered through fertigation with a composition (in mmol $L^{-1}$) of 11.0 N-$NO_3^-$, 1.5 S, 1.0 P, 6.0 K, 4.0 Ca, 2.0 Mg, 1.5 N-$NH_4^+$ and (in µmol $L^{-1}$) 20.0 Fe, 9.0 Mn, 0.3 Cu, 1.6 Zn, 20 B and 0.3 Mo with an electrical conductivity of 1.8 dS $m^{-1}$ and a pH of 6.2. Fertigation was performed once per day. When more than one irrigation event per day was necessary, plants were fertigated in the morning followed by irrigation event(s) with plain water until the end of the day. This strategy was used to avoid a buildup of salts in the rhizosphere.

*2.4. Plant growth measurement, yield and yield components*

At day 57 after transplanting (DAT; 28 September) the plant height and number of leaves per plant were recorded on 15 plants per experimental unit. Furthermore, fully ripe fruits were harvested at 72 DAT (13 October) and continued until the end of the experiment. During the harvest period, the number of fruits, mean weight and marketable yield were recorded on twenty plants located at the central part of each experimental unit. The marketable yield was expressed in kg $m^{-2}$. At the end of the experiment (14 November, 104 DAT) 15 plants per plot were separated into stems and leaves and their tissues were dried at 80 ºC for 72 h until they reached a constant weight to determine the corresponding dry biomasses. Dry biomass was equal to the sum of leaves and stems and was expressed in g $m^{-2}$.

*2.5. Collection of samples and fruit quality assessment*

The fruits of the third truss were analyzed for quality parameters. Equatorial and meridian diameters (mm) were determined at harvest and used to calculate the fruit shape index. Tomato fruits was excised and homogenized in a Waring blender (2 L capacity) for 1 min under low speed to avoid foaming. Part of the homogenate was filtered through double cheesecloth and the total soluble solids (TSS) content and also juice pH of the filtered liquid were measured on a digital Atago N1 refractometer (Atago Co. Ltd., Tokyo, Japan) and a pH meter (HI-9023; Hanna Instruments, Padova, Italy), respectively. The rest of the fresh homogenate samples were transferred to 50 ml falcon tubes, instantly dip-frozen in liquid nitrogen, and then stored at -80 °C for further chemical analyses. The fruits dry matter percentage was also determined in triplicates as a percentage of fresh mass following fruit desiccation to constant weight in a forced-air oven at 80 °C for 72 h, and weighed using an analytical balance (Denver Instruments, Denver, Colorado, USA).

Two hundred milligrams of freeze dried fruits were extracted with distilled water and the hydrophilic fraction (HAA) was measured with the N,N-dimethyl-p-phenylenediamine (DMPD) method (Fogliano et al., 1999). The lipophilic fraction (LAA) was also extracted from freeze-dried fruits (200 mg) with methanol, and antioxidant activity of this extract was measured with the 2,2′-azinobis 3-ethylbenzothiazoline-6-sulfonic acid ABTS method (Pellegrini et al., 1999). The absorbance of the HAA and LAA solutions was measured at 505 and 734 nm, respectively. HAA and LAA were expressed as mmol ascorbic acid and as mmol of Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) per 100 g of dry weight, respectively.

Lycopene content was determined as described by Sadler et al. (1990). Briefly, 5 g of homogenized sample was extracted adding 50 ml of a mixture of hexane/acetone/ethanol (2:1:1, v/v/v) for 30 min. The total lycopene content expressed in mg 100 $g^{-1}$ fresh weight was obtained by measuring the absorbance of the lycopene hexane fraction at 472 nm. Pure lycopene (Sigma, St. Louis, MO) was used for the preparation of calibration curves.

The total phenolic content in methanolic extracts was determined using the Folin-Ciocalteau procedure (Singleton et al., 1999) with gallic acid as a standard. A 100 µl aliquot of the supernatant was combined with 500 µl of Folin-Ciocalteau's reagent (Sigma Aldrich Inc, St Louis, MO, USA) and 400 µl of 7.5% sodium carbonate/water (w/v). Absorption was measured after 30 min at 765 nm using a UV-Vis spectrophotometer, and the result was expressed as mg gallic acid (Sigma Aldrich Inc, St Louis, MO, USA) per 100 g dry weight.

The total ascorbic acid defined as ascorbic acid and dehydroascorbate acid was also assessed by spectrophotometric detection. The assay is based on the reduction of $Fe^{3+}$ to $Fe^{2+}$ by ASA and the spectrophotometric detection of $Fe^{2+}$ complexes with 2,2-dipyridyl (Kampfenkel et al., 1995). The absorbance of the solution was measured at 525 nm, and the results were expressed as mg ascorbic acid on 100 g fresh weight.

*2.6. Net photosynthesis measurement*

At 90 DAT (31 October), the leaf net $CO_2$ assimilation rate ($A_{CO2}$; µmol $CO_2$ $m^{-2}$ $s^{-1}$) was measured with a portable gas exchange analyzer (LCA-4; ADC BioScientific Ltd., UK) equipped with a broadleaf chamber (cuvette window area of 6.25 $cm^2$). This physiological measurement was carried out within 2 h across solar noon (*i.e.* between 11.00 and 13.00) on the youngest fully expanded leaves, using six replicates for each treatment. Photosynthetically active radiation, relative humidity and carbon dioxide concentrations were set at ambient values (600 ± 30 µmol photons $m^{-2}$ $s^{-1}$, 50 ± 5% and 366 ± 8 ppm, respectively) and the flow rate of air was 400 ml $s^{-1}$.

*2.7. Mineral content analysis*

The total nitrogen concentration of the leaf samples was determined by the Kjeldahl method (Bremner, 1965), following mineralization with sulphuric acid (96%, Carlo Erba Reagents, Milan, Italy) in the presence of potassium sulfate and low concentration of copper catalyst. For the P, K, Ca, Mg and Na analysis, 250 mg of dried plant tissues (leaf and fruit) were extracted in 50 ml of ultrapure water (Milli-Q, Merck Millipore, Darmstadt, Germany) using a shaking water bath (ShakeTemp SW22, Julabo, Seelbach, Germany) at 80 ºC for 10 min. The mixture was centrifuged at 6000 rpm for 10 min (R-10M, Remi Elektrotechnik Limited, India), then filtered through a 0.20 µm filter paper (Whatman International Ltd., Maidstone, U.K.), as described previously by Rouphael et al. (2017b). Potassium, Ca, Mg and Na were separated by ion chromatography (ICS-3000, Dionex, Sunnyvale, CA, USA) and quantified through an electrical conductivity detector. Chromatographic separation was achieved in isocratic mode on an IonPac CS12A analytical column (4×250 mm, Dionex, Corporation) equipped with an IonPac CG12A precolumn (4×250 mm, Dionex, Corporation) and a self-regenerating suppressor CERS500 (4 mm, Dionex, Corporation). The Phosphorus content was also measured through ion chromatography coupled to a conductivity detector. A IonPac ATC-HC anion trap (9×75 mm), and a AS11-HC analytical column (4×250 mm) equipped with an AG11-HC precolumn (4×50 mm) and a self-regenerating suppressor AERS500 (4 mm) were used for separation.

*2.8. Statistical analysis of data*

Experimental data were subjected to analysis of variance (two way-ANOVA) using the software package SPSS 10 for Windows 2001. To separate treatment means within each measured parameter, the Duncan's Multiple Range Test was performed at $P \leq 0.05$. Principal component analysis (PCA) was performed on fruit morphometric components, mineral profile and juice phytochemical parameters to depict relationships among treatments and variables and also to individuate quality traits that were most effective in discriminating between cultivars and biostimulant application. PCA is a multivariate statistical method aimed to summarize and extract trends when several variables are used, by formulating new variables (*i.e.* Principal Components; PCs) correlated to the original ones (Lawless and Heymann, 2010). The PCs with eigenvalues greater than 1 were selected (Dunteman, 1989), and loadings greater than |0.6| indicated significant correlations between the original variables and the extracted components (Matus et al., 1996). The PCA outputs include variable loading to each selected component and treatment component scores. Analyses and calculations were carried out using the appropriate functions within SPSS 10 (SPSS Inc., USA).

## 3. Results and discussion

*3.1. Growth and yield responses*

Plant height and leaf number before the beginning of fruit harvest as well as shoot biomass and marketable yield were influenced by cultivar and biostimulant treatments with no significant cultivar × biostimulant interaction, whereas the mean fruit mass and fruit number incurred significant cultivar × biostimulant interaction (Table 1). Our data indicated that PH biostimulant application at 5.0 ml $L^{-1}$ enhanced plant height and leaf number by 14.3% and 21.8%, respectively, compared to PH applications at 2.5 ml $L^{-1}$ and untreated plants. Shoot biomass demonstrated a tendency for increase in response to PH biostimulant application rate; however, increase was

significant only at 5.0 ml $L^{-1}$, at which rate shoot biomass was higher by 19.6% over untreated plants, while no significant difference was found between the 2.5 and 5.0 ml $L^{-1}$ rates (Table 1). Similarly to the effects on plant growth parameters, the mean marketable yield in PH-treated plants at 5.0 ml $L^{-1}$ was significantly higher than that obtained from tomato plants treated at 2.5 ml $L^{-1}$ or untreated plants, with respective increases by 12.3% and 21.4% (Table 1). Interestingly, the higher marketable production observed on plants treated with the legume-derived PH biostimulant at 5.0 ml $L^{-1}$ and to a lesser extent at 2.5 ml $L^{-1}$, in comparison to the untreated plants, was due to an increase in the number of fruits per plant in the small fruit size cultivar Akyra and to a higher fruit weight in the medium fruit size cultivar Sir Elyan (Table 1). Furthermore, irrespective of the biostimulant treatment our results showed that Akyra was characterized by a more vigorous vegetative growth (*i.e.* higher plant height, leaf number and shoot biomass) in comparison to Sir Elyan, whereas the later cultivar demonstrated higher marketable yield (Table 1).

The increase in yield and growth responses of tomato plants has been reported in limited research studies testing the action of PHs under greenhouse conditions (Colla et al., 2015). These biostimulant effects on growth and crop productivity appear to be distinct from the nutritional effect of fertilizer (*i.e.*, N; Calvo et al., 2014). Our results on the foliar application of plant-derived PH at 5.0 ml $L^{-1}$ were consistent with the findings of Colla et al. (2014) who demonstrated that increasing the concentration of commercial legume-derived PH Trainer®, also used in the current experiment, from 0 to 10 ml $L^{-1}$ elicited significant increase in four weeks old tomato shoot, root and total biomass amounting to 19.5%, 27.5%, and 20.5%, respectively. The typical stimulation short-time effect that has been previously observed in PH-treated tomato plants (Parrado et al., 2008; Colla et al., 2014) was confirmed in our long period trial when the plant reached maturity. In fact, foliar application of legume-derived PH at 5.0 ml $L^{-1}$ increased marketable yield of Akyra and Sir Elyan by modulating yield components differently depending on cultivars (higher number of fruits in Akyra as opposed to increase of fruit mean weight in Sir Elyan).

A putative mechanism behind the stimulation of crop performance observed in response to foliar applications of legume-derived PH at 5.0 ml $L^{-1}$ could involve the augmented presence of signaling molecules, such as soluble peptides, oligopeptides and free amino acids, which are typical components of Trainer® (Colla et al., 2015). The low-molecular size peptides as well as free amino acids are easily absorbed by both leaves and roots and can act as signaling compounds in the regulation of plant growth and development, promoting endogenous phytohormonal biosynthesis (Cavani and Ciavatta, 2007). This was also confirmed by several authors (Colla et al., 2014; Matsumiya and Kubo, 2011; Ugolini et al., 2015) who demonstrated that leaf/root applications of plant-derived PHs containing amino acids, carbohydrates and 'root hair promoting peptides' exhibited auxin- and in some cases gibberellin-like activity, thus promoting plant growth, stimulating flowering, fruit setting and growth leading to a better yield.

*3.2. Net $CO_2$ assimilation rate, and leaf nutrient composition*

A significant effect of legume-derived PH application was observed on net leaf $CO_2$ assimilation rate ($A_{CO2}$; Fig. 1). $A_{CO2}$ was higher by 20.7% in the biostimulant treatment under both concentrations (avg. 11.7 μmol $CO_2$ $m^{-2}$ $s^{-1}$) compared to untreated tomato plants (9.7 μmol $CO_2$ $m^{-2}$ $s^{-1}$; Fig. 1).

Neither cultivar nor biostimulant treatment had a significant effect on total N, S and Ca concentration in leaves (avg. 30.7, 7.7 and 27.8 g $kg^{-1}$ dw, respectively; Table 2). Irrespective of biostimulant treatment, K and Na concentrations in leaf tissue were more pronounced in Akyra compared to Sir Elyan (Table 2). The PH biostimulant treatment at 5.0 ml $L^{-1}$, averaged over cultivars, affected the P and Mg concentrations in leaf tissue, which were higher by 27.6% and 32.9% on average than respective concentrations in PH 2.5 ml $L^{-1}$ and control treatments. Moreover, the highest K concentration was also observed in response to the 5.0 ml $L^{-1}$ treatment, though difference with the 2.5 ml $L^{-1}$ treatment was non-significant (Table 2).

It is well established that the amino acid and peptide components typical of Trainer® improve the plant's photosynthetic activity and subsequently lead to better yields (Colla et al., 2015). The improved functioning of the photosynthetic apparatus with both concentrations of the PH biostimulant could be associated to a better uptake and translocation of Mg. In fact, Mg is an essential macronutrient for plant growth and development and is involved in a wide range of biochemical and physiological activities, including pigment synthesis, energy metabolism and photosynthetic carbon fixation, while it forms the core component of chlorophylls' porphyrin ring (Gransee and Führs, 2013; Hermans et al., 2011; Kumar et al., 2015).

In addition to improved photosynthesis, legume-derived PH especially at 5.0 ml $L^{-1}$ influenced the capacity of tomato plants to absorb, translocate and accumulate macronutrients in leaf tissue. In the current experiment, tomato plants treated with PHs were able to maintain a better nutritional status, evidenced as higher P, K and Mg concentration in response to the 5.0 ml $L^{-1}$ application rate. The enhanced nutritional status has often been associated with the ability of plant-derived PH (e.g. legume-seeds hydrolysate or degraded soybean meal products; Colla et al., 2014; Matsumiya and Kubo, 2011) to modify the growth and morphology (*i.e.* architecture) of the root system, facilitating nutrient uptake as a consequence of the increased root absorbing area (Ertani et al., 2013; Rouphael et al., 2017b). A possible mechanism involved in the increase of root hair numbers and length could be related to the stimulation of auxin production by the soluble peptides present in the plant-derived PH Trainer®. Moreover, in a recent study, Ertani et al. (2017) demonstrated how an alfalfa-based PH tested on tomato plants at two different concentrations (0.1 and 1 ml $L^{-1}$) induced higher expression of nutrient transporters (NTP2, SULTR2, PT2), and elicited higher foliar accumulation of mineral elements, in particular P and K. The joint action of biostimulant-mediated increase of root surface area and nutrient transporter activity may explain the enhanced uptake of P, K, and Mg in leaves of tomato plants treated with plant-derived PH Trainer®.

*3.3. Fruit quality characteristics*

Whereas an overall increase in tomato yield was observed in biostimulant-treated plants, improving fruit quality by foliar applications of legume-derived PH is a challenging and pending issue as increased demand for horticultural commodities of premium flavor quality has characterized consumer behavior in recent years. In the current study, both cultivar and biostimulant application significantly affected the total soluble solids (TSS) content, whereas the fruit shape index (SI), dry matter content (DM) and juice pH were affected by cultivar only (Table 3).

The Sir Elyan cultivar showed a lower fruit SI, dry matter content and juice pH in comparison to the Akyra cultivar (Table 3). The lack of changes between treated and untreated plants on fruit SI could be expected since it constitutes an inherent quality trait linked to tomato fruit morphology, governed predominantly by genetic material (genotype), and little affected by environmental or cultural practices (*i.e.* biostimulant application) (Kyriacou et al., 2017). Concerning sugar content, which is a key quality attribute for consumer satisfaction (Kader, 2002), our results showed that highest juice TSS content was obtained in PH-treated plants at either concentration (Table 3). These findings are consistent with previous work in which treatment with two natural biostimulants: one derived from enzymatic hydrolysis of alfalfa plants (applied at 25 and 50 ml $L^{-1}$) and the other obtained by cool extraction of red grapes (applied at 50 and 100 ml $L^{-1}$), elicited an increase in the content of carbohydrates such as glucose and fructose of greenhouse chili pepper (*Capsicum chinensis* L.; Ertani et al., 2014).

It is well known that minerals command key roles in the human body's metabolism, thus human nutrition deficient in these bioactive constituents can result in nutritional disorders and expression of disease symptoms (Gharibzahedi and Jafari, 2017). Levander (1990) reported that fruits and vegetables contribute normally by 11%, 35%, 7% and 24% to the human dietary intake of total P, K, Ca and Mg, respectively. Taking into account the high annual per capita consumption of tomato, ranging from 31 kg in Europe (with the exception of Italy; 60 kg) to 42 kg in North America (FAOSTAT, 2014), the contribution of tomato fruit to human dietary intake of vitamins and especially minerals is highly significant. Among the macroelements studied, potassium was by far

the most abundant mineral constituent in the fruit of both tomato cultivars, followed by calcium, phosphorus, magnesium and finally sodium (Table 3). When averaged over biostimulant application, significant variation between the two tomato cultivars was recorded for all macronutrient and sodium, with the highest concentrations of K, Ca, Mg and Na recorded in Akyra, whereas an opposite was observed for P (Table 3). Concerning the influence of legume-derived PH application on fruit mineral profile, significant variation was recorded for K and Mg, while no significant differences among biostimulant applications were observed for P, Ca and Na (Table 3). Foliar application of legume-derived PH increased K and Mg significantly at the 5.0 ml $L^{-1}$ application rate, thereby enhancing the nutritional value of fresh-tomato fruits. Our current findings indicated that the increase in tomato fruit concentration (K and Mg) induced by the application of legume-derived PH at 5.0 ml $L^{-1}$ could be mediated through multiple mechanisms involving: (1) the presence of bioactive compounds in legume-derived PH Trainer® (soluble peptides, carbohydrates and free amino acids) which may have increased sink strength and influenced the movement of nutrient substrates, including minerals, within the plant (Calvo et al., 2014), (2) the greater uptake of minerals through a stimulation of root growth (Billard et al., 2014; Colla et al., 2014), and also (3) the higher expression of nutrient transporters in cell membranes (Ertani et al., 2017).

Antioxidant activities and biologically active compounds were also influenced by cultivar and biostimulant treatment with no significant cultivar × biostimulant application interaction (Table 4). The antioxidant activities, lycopene, total phenols and ascorbic acid contents recorded in the present study were cultivar-dependent with the highest values observed in the mini plum tomato Akyra (Table 4). The strong influence of genetic factors on antioxidant capacity and bioactive compounds has been previously demonstrated in greenhouse tomatoes (Fanasca et al., 2006 a,b). Antioxidant potential is a crucial parameter of the functional quality of vegetables and is usually linked to the synergetic effect of multiple low-molecular weight antioxidants (e.g., vitamin C and phenolic compounds) (Colonna et al., 2016). In the current experiment, the LAA and HAA of fresh tomato ranged from 0.28 to 1.23 mmol Trolox eq. 100 $g^{-1}$ dw and from 1.04 to 2.54 mmol ascorbic acid eq.

100 $g^{-1}$ dw, with the highest levels of LAA and HAA, averaged over cultivars, recorded in response to plant-derived PH applications at 5.0 ml $L^{-1}$. Contrary to total phenols content, the total ascorbic acid and lycopene contents were significantly influenced by biostimulant treatments. Compared to the fruit of untreated plants, lycopene content was increased by 34.9% and 18.0% in response to foliar application of commercial legume-derived PH delivered at 5.0 and 2.5 ml $L^{-1}$, respectively. Similarly, the total ascorbic acid in PH-treated plants at 5 ml $L^{-1}$ was significantly higher by 27.3% than that recorded in 2.5 ml $L^{-1}$ treated and untreated tomato plants (Table 4). These findings are in agreement with those of Ertani et al. (2014), who reported that ascorbic acid increased 2.5-fold in green chili pepper fruits treated with alfalfa-derived biostimulant at 25 ml $L^{-1}$, whilst it increased 1.28-fold in red chili pepper fruits following the application of red grape-derived biostimulant at 100 ml $L^{-1}$, in comparison to untreated plants.

The synthesis and accumulation of antioxidant compounds such as ascorbic and lycopene could be attributed to direct or indirect effects of biostimulant applications on biosynthesis and turnover of antioxidants in plant tissues. Plant-derived PH applications may have promoted the activity of specific enzymes (direct effects) involved in the antioxidant homeostasis in cells as reported by Ertani et al. (2014). Moreover, indirect effects such as the changes of K uptake and leaf coverage may have influenced the accumulation of antioxidants in fruits. Lycopene content was significantly correlated (Pearson's coefficient = 0.915; $p < 0.05$) to K concentration in the fruits (data not shown). The same type of correlation between lycopene and K concentration has been reported in tomatoes (Fanasca et al., 2006 a,b; Taber et al., 2008). Potassium is essential for the pyruvic kinase and acetic thiokinase enzymes that are directly involved in lycopene synthesis. Potassium may also affect lycopene synthesis through enhanced enzymatic activity in carbohydrate metabolism, providing the necessary substrates for terpenoid synthesis in tomato (Fanasca et al., 2006a; Taber et al., 2008). Similarly, ascorbic acid increased with increasing levels of K in several crops (Lester, 2006; Rouphael et al., 2012). Moreover, climate factors have an important role in antioxidant accumulation in tomato fruits. High fruit temperatures can destroy lycopene and slow lycopene

synthesis (Taber et al., 2008); similarly, ascorbic acid declined with increasing temperature in tomato fruits (Lester, 2006). The biostimulant-mediated increase in leaf number may have enhanced fruit shading resulting in a reduction of fruit temperature and thus an increase of antioxidant compounds. The incurred accumulation of these health-promoting metabolites, lycopene in particular, in response to legume-derived PH-applications could be considered an *added value* to tomato's contribution toward human health and longevity (Erba et al., 2013). In fact, several pre-clinical and clinical investigations have revealed that this natural pigment reduces the risk or progression of diseases associated with oxidative stress (Berman et al., 2015).

*3.4. Principal Component Analysis*

A comprehensive framework of biostimulant application and cultivar effects on morphometric and physicochemical quality characteristics of fresh tomato was obtained through Principal Component Analysis (PCA). The first two PCs were associated with Eigen values higher than one and explained cumulatively 93% of the total variance, with PC1 and PC2 accounting for 79.3% and 13.7%, respectively (Table 5). PC1 was highly and positively correlated with HAA, bioactive compounds (lycopene, total ascorbic acid and phenols), DM, TSS, K, Ca, Mg , juice pH and fruit SI; it was negatively correlated with fruit weight and P (Table 5). PC2 was positively correlated with LAA and negatively associated with Na content (Table 5). Moreover, the loading plot in Fig. 2A illustrates the correlation among variables, where two vectors with an angle lower than 90º are positively correlated and two vectors with an angle higher than 90º are negatively correlated. For instance, variation in TSS content was most closely aligned with lycopene, and variation in ascorbic acid was more strongly correlated to Mg content (narrower angle between the corresponding vectors) rather than LAA. The correlation between sugars (TSS) and total ascorbic acid could be expected since soluble hexoses are known precursors of ascorbate synthesis (Nagy, 1980).

The effectiveness of PCA plotting in genetic characterization and in preharvest and postharvest quality experiments has been documented in several scientific papers (Kyriacou et al., 2016,

Rouphael et al., 2016, 2017c). This was also the case in the present study, since PCA scores in Fig. 2B have introduced concerted information on the tomato quality traits in relation to cultivars and biostimulant application. The PC1 and PC2 score plot separates and categorizes biostimulant treatments and cultivars into four groups (quadrants). The upper left quadrant of the negative side of PC1 (Q1) included cultivar Sir Elyan treated with plant-derived PH at 5.0 ml $L^{-1}$ that delivered fruit of high weight and high P content (Fig 2B). A second group clustered on the positive side of PC1 (Q2) included Akyra treated with PH at both 2.5 and 5.0 ml $L^{-1}$, representing tomato fruits with premium quality (high LAA, lycopene, K and Mg contents). The lower left quadrant (Q3) depicted the treatments (Sir Elyan untreated or treated at 2.5 ml $L^{-1}$) of lowest quality traits. Finally, treatments in Q4 had the highest Na and fruit SI encompassing the untreated Akyra (Fig. 2B). Moreover, score plot clearly separates the two cultivars along PC1 with Akyra concentrating most of the flavor and functional quality traits while Sir Elyan stands out for its fruit weight and P content. The biostimulant applications are clustered mainly in respect to PC2, with the 5.0 ml $L^{-1}$ application on the positive side of PC2 that is characterized by improved size, flavor or functional quality attributes. Overall, the PCA outputs may provide the basis for a more in-depth approach to elucidate the influence of cultivars and biostimulant treatments on quality characteristics of greenhouse tomato.

## 4. Conclusions

The increasing pressure on vegetable farmers to intensify agricultural production while maintaining premium quality represents a strong stimulus for scientists to propose alternative sustainable technologies for the improvement of vegetable quality, in order to meet consumer demand. Greenhouse tomato crop performance and the configuration of fruit quality was analyzed in a multifactorial approach accounting for the effects of biostimulant treatments and cultivars. Foliar applications of legume-derived PH Trainer® at 5.0 ml $L^{-1}$ increased marketable yield of Akyra and Sir Elyan by modulating yield components (number or fruits and mean weight)

differently depending on cultivars. Application of the biostimulant, especially at the high dose, was capable of maintaining higher photosynthesis and a better nutritional status for the plants. Our results also demonstrated, that differences in sensory and functional fruit quality traits were identified between control and plant-derived PH treated tomato plants at 2.5 and 5.0 ml $L^{-1}$. Key quality attributes of greenhouse fresh tomato (TSS, LAA, HAA, total ascorbic acid lycopene, K and Mg contents) were improved by the applications of legume-derived PH Trainer®.

**Acknowledgements**

The authors are grateful to Dr. Alfonso Marilongo for his assistance in the greenhouse experiment and also to Dr. Antonio Pannico for the leaf gas exchange measurements.

**References**

Berman, J., Zorrilla-López, U., Farré, G., Zhu, C., Sandmann, G., Twyman, R.M., Capell, T., Christou, P., 2015. Nutritionally important carotenoids as consumer products. Phytochem. Rev. 14, 727–743.

Billard, V., Etienne, P., Jannin, L., Garnica, M., Cruz, F., Garcia-Mina, J.M., Yvin, J.C., Ourry, A., 2014. Two biostimulants derived from algae or humic acid induce similar responses in the mineral content and gene expression of winter oilseed rape (*Brassica napus* L.). J. Plant Growth Regul. 33, 305–316.

Botta, A., 2013. Enhancing plant tolerance to temperature stress with amino acids: an approach to their mode of action. Acta Hortic. 1009, 29–35.

Bramley, P.M., 2002. Regulation of carotenoids formation during tomato fruit ripening and development. J. Exp. Bot. 53, 2107–2113.

Bremner, J.M., 1965. Total nitrogen, in: Black, C.A., Evans, D.D., White, I.L., Ensminger, L.E., Clark, F.E. (Eds.), Methods of Soil Analysis. American Society of Agronomy, Madison, pp. 1149–11789.

Calvo, P., Nelson, L., Kloepper, J.W., 2014. Agricultural uses of plant biostimulants. Plant Soil 383, 3–41.

Cavani, L., Ciavatta, C., 2007. Attività biostimolante negli idrolizzanti proteici. L'Informatore Agrario 44, 46–52.

Cerdán, M., Sánchez-Sánchez, A., Oliver, M., Juárez, M., Sánchez-Andreu, J.J., 2009. Effect of foliar and root applications of amino acids on iron uptake by tomato plants. Acta Hortic. 830, 481–488.

Colla, G., Svecova, E., Rouphael, Y., Cardarelli, M., Reynaud, H., Canaguier, R., Planques, B., 2013. Effectiveness of a plant –derived protein hydrolysate to improve crop performances under different growing conditions. Acta Hortic. 1009, 175–179.

Colla, G., Rouphael, Y., Canaguier, R., Svecova, E., Cardarelli, M., 2014. Biostimulant action of a plant-derived protein hydrolysate produced through enzymatic hydrolysis. Fron. Plant Sci. 5, 1–6.

Colla, G., Nardi, S., Cardarelli, M., Ertani, A., Lucini, L., Canaguier, R., Rouphael, Y., 2015. Protein hydrolysates as biostimulants in horticulture. Sci. Hortic. 96, 28–38.

Colla, G., Rouphael, Y., 2015. Biostimulants in horticulture. Sci. Hortic. 196, 1–2.

Colla, G., Cardarelli, M., Bonini, P., Rouphael, Y., 2017. Foliar applications of protein hydrolysate, plant and seaweed extracts increase yield but differentially modulate fruit quality of greenhouse tomato. HortScience (In Press).

Colonna, E., Rouphael, Y., Barbieri, G., De Pascale, S., 2016. Nutritional quality of leafy vegetables harvested at two light intensities. Food Chem. 199, 702–710.

Duhamel, M., Vandenkoornhuyse, P., 2013. Sustainable agricolture: possible trajectories from mutualistic simbiosi and plant neodomestication. Trends Plant Sci. 11, 597–600.

du Jardin, P., 2015. Plant biostimulants: definition, concept, main categories and regulation. Sci. Hortic. 196, 3–14.

Dunteman, G.H., 1989. Principal Components Analysis. Sage publications, London.

Erba, D., Casiraghi, M.C., Ribas-Agustí, A., Cáceres, R., Marfà, O., Castellari, M., 2013. Nutritional value of tomatoes (*Solanum lycopersicum* L.) grown in greenhouse by different agronomic techniques. J. Food Comp. Anal. 31, 245–51.

Ertani, A., Cavani, L., Pizzeghello, D., Brandellero, E., Altissimo, A., Ciavatta, C., Nardi, S., 2009. Biostimulant activities of two protein hydrolysates on the growth and nitrogen metabolism in maize seedlings. J. Plant. Nutr. Soil. Sci. 172, 237–244.

Ertani, A., Schiavon, M., Muscolo, A., Nardi, S., 2013. Alfalfa plant-derived biostimulant stimulate short-term growth of salt stressed *Zea mays* L. plants. Plant Soil 364, 145–158.

Ertani, A., Pizzeghello, D., Francioso, O., Sambo, P., Sanchez-Cortes, S., Nardi, S., 2014. *Capsicum chinensis* L. growth and nutraceutical properties are enhanced by biostimulants in a long-term period: chemical and metabolomic approaches. Fron. Plant Sci. 5, 1–12.

Ertani, A., Schiavon, M., Nardi, S., 2017. Transcriptome-wide identification of differentially expressed genes in *Solanum lycopersicon* L. in response to an alfalfa-protein hydrolysate using microarrays. Front. Plant Sci. 8, 1159.

Fanasca, S., Colla, G., Maiani, G., Venneria, E., Rouphael, Y., Azzini, E., Saccardo, F., 2006a. Changes in antioxidant content of tomato fruits in response to cultivar and nutrient solution composition. J. Agric. Food Chem. 54, 4319–4325.

Fanasca, S., Colla, G., Rouphael, Y., Saccardo, F., Maiani, G., Venneria, E., Azzini, E., 2006b. Evolution of nutritional value of two tomato genotypes grown in soilless culture as affected by macrocation proportions. Hort Sci. 441, 1584–1588.

FAOSTAT 2014. Production. www.faostat.fao.org.

Feitosa de Vasconcelos, A.C., Zhang, X., Ervin, E.H., de Castro Kieh, J., 2009. Enzymatic antioxidant responses to biostimulants in maize and soybean subjected to drought. Sci. Agric. (Piracicaba, Braz.) 66, 395–402.

Fogliano, V., Verde, V., Randazzo, G., Ritieni, A., 1999. Method for measuring antioxidant activity and its application to monitoring the antioxidant capacity of wines. J. Agric. Food Chem. 47, 1035–1040.

Gharibzahedi, S.M.T., Jafari, S.M., 2017. The importance of minerals in human nutrition: Bioavailability, food fortification, processing effects and nanoencapsulation. Trends Food Sci. Technol. 62, 119–132.

Gransee, A., Führs, H., 2013. Magnesium mobility in soils as a challenge for soil and plant analysis, magnesium fertilization and root uptake under adverse growth conditions. Plant Soil 368, 5–21.

Hermans, C., Chen, J., Coppens, F., Inze, D., Verbruggen, N., 2011. Low magnesium status in plants enhances tolerance to cadmium exposure. New Phytol. 192, 428–436.

Kader, A.A., 2002. Postharvest Technology of Horticultural Crops, University of California, Division of Agriculture and Natural Resources Publication 3311, pp. 535.

Kampfenkel, K., Montagu, M.V., Inzè, D., 1995. Extraction and determination of ascorbate and dehydroascorbate from plant tissue. Ann. Rev. Biochem. 225, 165–167.

Kumar, P., Lucini, L., Rouphael, Y., Cardarelli, M., Kalunke, R.M., Colla, G., 2015. Insight into the role of grafting and arbuscular mycorrhiza on cadmium stress tolerance in tomato. Front. Plant Sci. 6, 477.

Kyriacou, M.C., Soteriou, G.A., Rouphael, Y., Siomos, A.S., Gerasopoulos, D., 2016. Configuration of watermelon fruit quality in response to rootstock-mediated harvest maturity and postharvest storage. J. Sci. Food Agric. 96, 2400–2409.

Kyriacou, M.C., Rouphael, Y., Colla, G., Zrenner, R.M., Schwarz, D., 2017. Vegetable grafting: The implications of a growing agronomic imperative for vegetable fruit quality and nutritive value. Fron. Plant Sci. 8, 741.

Lawless, H., Heymann, H., 2010. Sensory Evaluation of Food: Principles and Practices. Springer, New York, NY.

Lester, G. E., 2006. Environmental regulation of human health nutrients (ascorbic acid, carotene, and folic acid) in fruits and vegetables. HortSci. 41, 59-64.

Levander, O.A., 1990. Fruit and vegetable contributions to dietary mineral intake in human health and disease. HortSci. 25, 1486–1488.

Lucini, L., Rouphael, Y., Cardarelli, M., Canguier, R., Kumar, P., Colla, G., 2015. The effect of a plant-derived biostimulant on metabolic profiling and crop performance of lettuce grown under saline conditions. Sci. Hortic. 182, 124–133.

Matsumiya, Y., Kubo, M., 2011. Soybean peptide: novel plant growth promoting peptide from soybean, in: El-Shemy H. (Ed.), Soybean and Nutrition. Rijeka: In Tech Europe Publisher, pp. 215–230.

Matus, L.M., Gonzales, G., del Poso, A., 1996. Evaluation of phenotypic variation in a Chilian collection of garlic (*Allium sativum* L.) clones using multivariate analysis. Plant Genet. Resour. Newslett. 117, 31–36.

Nagy, S., 1980. Vitamin C contents of citrus fruit and their products: a review. J. Agric. Food Chem. 28, 8–18.

Parrado, J., Bautista, J., Romero, E.J., García-Martínez, A.M., Friaza, V., Tejada, M., 2008. Production of a carob enzymatic extract: potential use as a biofertilizer. Bioresource Technol. 99, 2312–2318.

Pellegrini, N., Re, R., Yang, M., Rice-Evans, C., 1999. Screening of dietary carotenoids and carotenoid-rich fruit extracts for antioxidant activities applying 2,2'-azinobis(3-ethylenebenzothiazoline-6- sulfonic acid radical cation decolorization assay. Meth. Enzymol. 299, 379–384.

Rouphael, Y., Cardarelli, M., Bassal, A., Leonardi, C., Giuffrida, F., Colla, G., 2012. Vegetable quality as affected by genetic, agronomic and environmental factors. J. Food Agric. Environ. 10, 680-688.

Rouphael, Y., Bernardi, J., Cardarelli, M., Bernardo, L., Kane, D., Colla, G., Lucini, L., 2016. Phenolic compounds and sesquiterpene lactones profile in leaves of nineteen artichoke cultivars. J. Agric. Food Chem. 64, 8540−8548.

Rouphael, Y., Cardarelli, M., Bonini, P., Colla, G., 2017a. Synergistic action of a microbial-based biostimulant and a plant derived-protein hydrolysate enhances lettuce tolerance to alkalinity and salinity. Front. Plant Sci. 8, 131.

Rouphael, Y., De Micco, V., Arena, C., Raimondi, G., Colla, G., De Pascale, S., 2017b. Effect of *Ecklonia maxima* seaweed extract on yield, mineral composition, gas exchange and leaf anatomy of zucchini squash grown under saline conditions. J. Appl. Phycol. 29, 459–470.

Rouphael, Y., Colla, G., Graziani, G., Ritieni, A., Cardarelli, M., De Pascale, S., 2017c. Phenolic compositions, antioxidant activity and mineral profile in two seed-propagated artichoke cultivars as affected by microbial inoculants and planting time. Food Chem. 234, 10–19.

Sadler, G., Davis, J., Dezman, D., 1990. Rapid extraction of lycopene and β-carotene from reconstituted tomato paste and pink grapefruit homogenates. J. Food Sci. 55, 1460–1461.

Schaafsma, G., 2009. Safety of protein hydrolysates, fractions thereof and bioactive peptides in human nutrition. Eur. J. Clin. Nutr. 63, 1161–1168.

Schiavon, M., Ertani, A., Nardi, S., 2008. Effects of an alfaalfa protein hydrolysate on the gene expression and activity of enzymes of TCA cycle and N metabolism in *Zea mays* L. J. Agric. Food Chem. 56, 11800–11808.

Singleton, V.L., Orthofer, R., Lamuela-Raventos, R.M., 1999. Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin–Ciocalteu reagent. Meth. Enzymol. 299, 152–178.

Ugolini, L., Cinti, S., Righetti, L., Stefan, A., Matteo, R., D'Avino, L., Lazzeri, L., 2015. Production of an enzymatic protein hydrolyzate from defatted sunflower seed meal for potential application as a plant biostimulant. Ind Crops Prod. 75, 15–23.

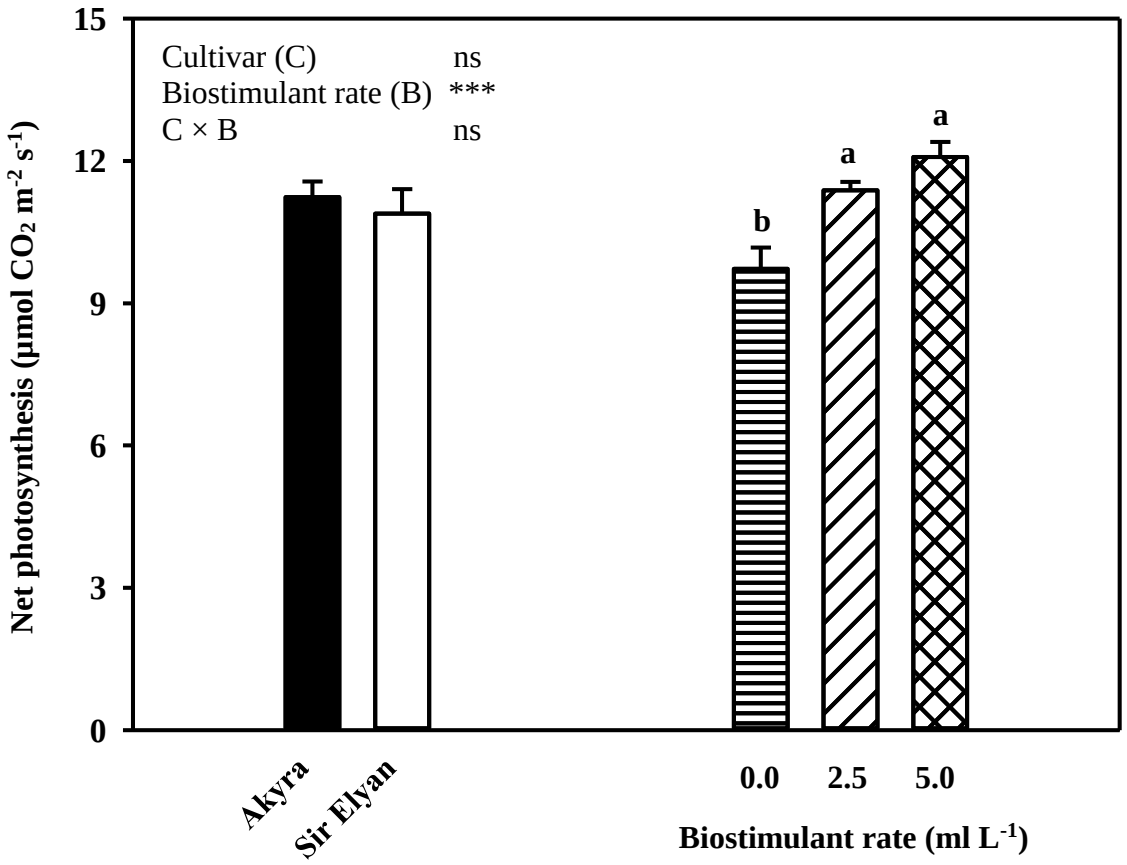



Figure 1. Mean effects of cultivar and biostimulant rate on net $CO_2$ assimilation rate of tomato leaves. The values are means of three replicates. Vertical bars indicate ± SE of means.

Table 1 - Analysis of variance and mean comparisons for plant height and leaf number before the beginning of fruit harvest (57 days after transplanting), and total dry biomass of shoots, marketable yield and yield components of two tomato cultivars foliarly sprayed with three biostimulant rates.

| Source of variance | Plant height (m) | Leaf number (n. plant$^{-1}$) | Dry shoot biomass (g m$^{-2}$) | Marketable yield (kg m$^{-2}$) | Marketable fruit | |
|---|---|---|---|---|---|---|
| | | | | | Mean mass (g fruit$^{-1}$) | Number (no. m$^{-2}$) |
| Cultivar | *** | *** | *** | ** | *** | *** |
| Biostimulant rate | *** | ** | * | ** | *** | ns |
| Cultivar × Biostimulant rate | ns | ns | ns | ns | *** | * |
| Cultivar | | | | | | |
| Akira | 2.5 a | 58.1 a | 438.9 a | 10.1 b | 31.7 b | 319.5 a |
| Sir Elyan | 2.0 b | 43.4 b | 317.4 b | 11.4 a | 84.6 a | 134.3 b |
| Biostimulant rate (ml L$^{-1}$) | | | | | | |
| 0.0 | 2.1 b | 46.7 b | 341.0 b | 9.8 b | 52.6 c | 217.2 |
| 2.5 | 2.1 b | 47.9 b | 385.6 ab | 10.6 b | 58.0 b | 228.6 |
| 5.0 | 2.4 a | 57.6 a | 407.8 a | 11.9 a | 63.8 a | 234.9 |
| Cultivar × Biostimulant rate | | | | | | |
| Akira without biostimulant | 2.4 | 54.5 | 391.5 | 9.1 | 31.1 d | 293.4 b |
| Akira with biostimulant at 2.5 ml L$^{-1}$ | 2.4 | 56.0 | 450.3 | 10.5 | 31.6 d | 330.8 a |
| Akira with biostimulant at 5.0 ml L$^{-1}$ | 2.6 | 63.8 | 474.9 | 10.8 | 32.3 d | 334.2 a |
| Sir Elyan without biostimulant | 1.9 | 38.9 | 290.7 | 10.5 | 74.1 c | 141.0 c |
| Sir Elyan with biostimulant at 2.5 ml L$^{-1}$ | 1.8 | 39.9 | 321.0 | 10.7 | 84.4 b | 126.4 c |
| Sir Elyan with biostimulant at 5.0 ml L$^{-1}$ | 2.1 | 51.3 | 340.8 | 12.9 | 95.4 a | 135.6 c |

[a]ns,*,**, *** Nonsignificant or significant at $P \leq 0.05$, 0.01, and 0.001, respectively. Different letters within each column indicate significant differences according to Duncan's multiple-range test ($P = 0.05$).

Table 2 - Analysis of variance and mean comparisons for macronutrient content in leaves and fruits of two tomato cultivars foliarly sprayed with three biostimulant rates.

| Source of variance | Mineral composition (g $kg^{-1}$ dw) | | | | | | |
|---|---|---|---|---|---|---|---|
| | N | P | S | K | Ca | Mg | Na |
| Cultivar | ns | ns | ns | *** | ns | ns | ** |
| Biostimulant rate | ns | * | ns | * | ns | ** | ns |
| Cultivar × Biostimulant rate | ns | ns | ns | ns | ns | ns | ns |
| | | | | | | | |
| Cultivar | | | | | | | |
| Akira | 31.2 | 2.6 | 7.8 | 40.6 a | 27.5 | 7.9 | 3.5 a |
| Sir Elyan | 30.3 | 2.6 | 7.6 | 29.2 b | 28.1 | 8.2 | 2.3 b |
| | | | | | | | |
| Biostimulant rate (ml $L^{-1}$) | | | | | | | |
| 0.0 | 29.1 | 2.2 b | 6.8 | 32.8 b | 25.6 | 6.8 b | 3.1 |
| 2.5 | 30.7 | 2.5 b | 8.2 | 34.4 ab | 28.2 | 7.8 b | 2.8 |
| 5.0 | 32.5 | 3.0 a | 8.2 | 37.5 a | 29.7 | 9.7 a | 2.8 |
| | | | | | | | |
| Cultivar × Biostimulant rate | | | | | | | |
| Akira without biostimulant | 28.7 | 2.2 | 7.0 | 38.8 | 25.7 | 6.8 | 3.8 |
| Akira with biostimulant at 2.5 ml $L^{-1}$ | 31.7 | 2.5 | 8.2 | 39.0 | 27.6 | 7.3 | 3.0 |
| Akira with biostimulant at 5.0 ml $L^{-1}$ | 33.2 | 3.2 | 8.3 | 44.0 | 29.2 | 9.6 | 3.6 |
| Sir Elyan without biostimulant | 29.5 | 2.3 | 6.6 | 26.8 | 25.4 | 6.7 | 2.4 |
| Sir Elyan with biostimulant at 2.5 ml $L^{-1}$ | 29.7 | 2.5 | 8.2 | 29.7 | 28.8 | 8.2 | 2.6 |
| Sir Elyan with biostimulant at 5.0 ml $L^{-1}$ | 31.8 | 2.8 | 8.1 | 31.0 | 30.2 | 9.8 | 1.9 |

[a]ns,*,**, *** Nonsignificant or significant at $P \leq 0.05$, 0.01, and 0.001, respectively. Different letters within each column indicate significant differences according to Duncan's multiple-range test (P = 0.05).

Table 3 - Analysis of variance and mean comparisons for shape index, dry matter, total soluble solids, juice pH and mineral composition in fruits of two tomato cultivars foliarly sprayed with three biostimulant rates.

| Source of variance | Shape index | Dry matter (%) | Total soluble solids (°Brix) | pH | Mineral composition (g $kg^{-1}$ dw) | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | P | K | Ca | Mg | Na |
| Cultivar | *** | ** | *** | * | * | ** | *** | *** | * |
| Biostimulant rate | ns | ns | * | ns | ns | * | ns | ** | ns |
| Cultivar × Biostimulant rate | ns | ns | ns | ns | ns | ns | ns | ns | ns |
| Cultivar | | | | | | | | | |
| Akira | 0.75 a | 6.84 a | 4.90 a | 4.48 a | 4.65 b | 35.15 a | 7.30 a | 1.34 a | 0.86 a |
| Sir Elyan | 0.68 b | 6.16 b | 4.02 b | 4.40 b | 6.68 a | 31.78 b | 4.54 b | 0.92 b | 0.66 b |
| Biostimulant rate (ml $L^{-1}$) | | | | | | | | | |
| 0.0 | 0.72 | 6.46 | 4.22 b | 4.42 | 6.18 | 32.28 b | 5.59 | 0.99 b | 0.87 |
| 2.5 | 0.71 | 6.50 | 4.50 a | 4.43 | 5.47 | 32.44 b | 5.97 | 1.14 ab | 0.73 |
| 5.0 | 0.72 | 6.55 | 4.67 a | 4.46 | 5.34 | 35.67 a | 6.19 | 1.27 a | 0.68 |
| Cultivar × Biostimulant rate | | | | | | | | | |
| Akira without biostimulant | 0.77 | 6.81 | 4.63 | 4.44 | 4.73 | 34.34 | 7.35 | 1.22 | 1.06 |
| Akira with biostimulant at 2.5 ml $L^{-1}$ | 0.72 | 6.94 | 4.93 | 4.48 | 3.95 | 34.37 | 7.24 | 1.35 | 0.80 |
| Akira with biostimulant at 5.0 ml $L^{-1}$ | 0.75 | 6.79 | 5.13 | 4.52 | 5.27 | 36.73 | 7.30 | 1.46 | 0.71 |
| Sir Elyan without biostimulant | 0.66 | 6.12 | 3.80 | 4.40 | 7.64 | 30.21 | 3.84 | 0.77 | 0.68 |
| Sir Elyan with biostimulant at 2.5 ml $L^{-1}$ | 0.71 | 6.06 | 4.07 | 4.38 | 6.99 | 30.52 | 4.69 | 0.93 | 0.66 |
| Sir Elyan with biostimulant at 5.0 ml $L^{-1}$ | 0.68 | 6.32 | 4.20 | 4.41 | 5.41 | 34.62 | 5.08 | 1.07 | 0.65 |

[a]ns,*,**, *** Nonsignificant or significant at $P \leq 0.05$, 0.01, and 0.001, respectively. Different letters within each column indicate significant differences according to Duncan's multiple-range test (P = 0.05).

Table 4 - Analysis of variance and mean comparisons for lipophilic and hydrophilic antioxidant activities, lycopene, total phenols and total ascorbic acid in fruits of two tomato cultivars foliarly sprayed with three biostimulant rates.

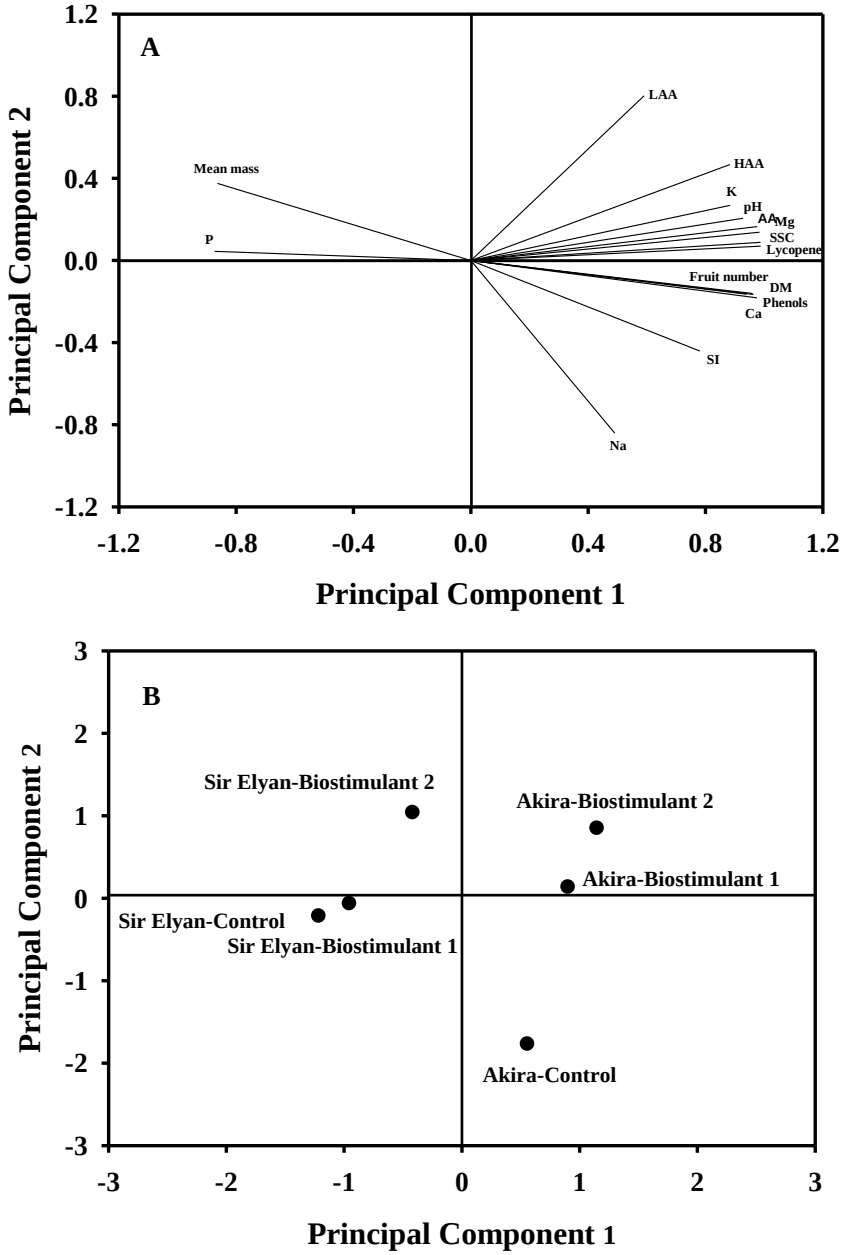
| Source of variance | Antioxidant capacity | | Lycopene (mg 100 $g^{-1}$ fw) | Total phenols (mg gallic acid eq. 100 $g^{-1}$ dw) | Total ascorbic acid (mg 100 $g^{-1}$ fw) |
|---|---|---|---|---|---|
| | Lipophilic (mmol Trolox eq. 100 $g^{-1}$ dw) | Hydrophilic (mmol ascorbic acid eq. 100 $g^{-1}$ dw) | | | |
| Cultivar | * | ** | *** | *** | *** |
| Biostimulant rate | *** | * | *** | ns | *** |
| Cultivar × Biostimulant rate | ns | ns | ns | ns | ns |
| Cultivar | | | | | |
| Akira | 0.63 a | 1.83 a | 69.2 a | 27.0 a | 109.1 a |
| Sir Elyan | 0.45 b | 1.17 b | 40.7 b | 18.3 b | 69.9 b |
| Biostimulant rate (ml $L^{-1}$) | | | | | |
| 0.0 | 0.24 c | 1.17 b | 46.7 c | 22.5 | 76.9 b |
| 2.5 | 0.58 b | 1.42 b | 55.1 b | 22.7 | 87.1 b |
| 5.0 | 0.80 a | 1.89 a | 63.0 a | 22.9 | 104.4 a |
| Cultivar × Biostimulant rate | | | | | |
| Akira without biostimulant | 0.21 | 1.33 | 63.5 | 25.6 | 98.2 |
| Akira with biostimulant at 2.5 ml $L^{-1}$ | 0.76 | 1.93 | 69.6 | 27.8 | 106.7 |
| Akira with biostimulant at 5.0 ml $L^{-1}$ | 0.91 | 2.21 | 74.5 | 27.6 | 122.3 |
| Sir Elyan without biostimulant | 0.27 | 0.91 | 30.0 | 19.3 | 55.6 |
| Sir Elyan with biostimulant at 2.5 ml $L^{-1}$ | 0.39 | 1.02 | 40.6 | 17.5 | 67.5 |
| Sir Elyan with biostimulant at 5.0 ml $L^{-1}$ | 0.69 | 1.57 | 51.4 | 18.1 | 86.6 |

[a]ns,*,**, *** Nonsignificant or significant at $P \leq 0.05$, 0.01, and 0.001, respectively. Different letters within each column indicate significant differences according to Duncan's multiple-range test (P = 0.05).

**Table 5.** Eigen values, relative and cumulative percentage of total variance, and correlation coefficients for each wheat trait with respect to the two principal components (PC1 and PC2).

| Principal components | PC1 | PC2 |
|---|---|---|
| Eigen value | 12.6 | 2.1 |
| Relative variance (%) | 79.3 | 13.7 |
| Cumulative variance (%) | 79.3 | 93.0 |
| | | |
| *Eigen vectors* | | |
| Lycopene | **0.988** | 0.070 |
| Soluble solids content | **0.988** | 0.088 |
| Mg | **0.984** | 0.137 |
| Total ascorbic acid | **0.977** | 0.165 |
| Ca | **0.976** | -0.182 |
| Dry matter percentage | **0.964** | -0.166 |
| Fruit number | **0.960** | -0.161 |
| Total phenolics | **0.940** | -0.164 |
| Juice pH | **0.928** | 0.206 |
| K | **0.884** | 0.268 |
| Hydrophilic antioxidant activity | **0.883** | 0.467 |
| P | **-0.873** | 0.044 |
| Fruit mean mass | **-0.864** | 0.375 |
| Shape index | **0.781** | -0.441 |
| Na | 0.491 | **-0.841** |
| Lipophylic antioxidant activity | 0.591 | **0.802** |

Boldface factor loadings indicate the most relevant characters for each principal component




9

10 Fig. 2. (A) Principal component loading plot and (B) scores of principal component
11 analysis of quality traits of greenhouse tomato as a function of biostimulant application
12 rate (control = 0.0 ml $L^{-1}$; 1 = 2.5 ml $L^{-1}$; 2 = 5.0 ml $L^{-1}$), and cultivars (Akyra and Sir
13 Elyan). LAA, lipophylic antioxidant activity; HAA, hydrophilic antioxidant activity;
14 AA, total ascorbic acid; TSS, total soluble solids; DM, dry matter; SI, shape index.