Foliar Applications of Protein Hydrolysate, Plant and Seaweed Extracts Increase Yield but Differentially Modulate Fruit Quality of Greenhouse Tomato

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Abstract. The current research aimed 1) at evaluating the effects of three biostimulants (legume-derived protein hydrolysate, PH; plant and seaweed extract, PE and SWE) on yield performance and nutritional quality, mineral profiling, antioxidant activities, lycopene, total phenols and ascorbic acid of greenhouse tomato (*Solanum lycopersicum* L.) under soil culture and 2) to assess the economic profitability of biostimulant applications. Plants were sprayed four times during the growing cycle with a solution containing 1, 3, and 3 mL·L⁻¹ of PE, SWE, and PH, respectively. Foliar applications of biostimulants improved the early and total marketable yield of fresh tomato. The increase of total yield by PE, SWE, and PH was 11.7%, 6.6% and 7.0%, respectively, in comparison with untreated plants. Legume-derived PH increased lycopene, total soluble solids, and K and Mg contents, thereby increasing the nutritional value of the fruits. The applications of SWE, and to a lesser degree PH, enhanced the Ca concentration in the fruit tissue. Our findings indicated that the three tested biostimulants, although they increased the total production cost, improved the nutrient status and yield performance of the crop to a level resulting in net economic benefits.

Modern agriculture is blamed for increasing environmental pollution. This is especially true for vegetable production systems where the soil fertilizer application has been often associated with an increase in nutrient pollution because of the limited mineral uptake by the shallow root system (Colla et al., 2015a). Therefore, in the coming few years, modern agriculture must meet the twin challenge of feeding the increasing global population while simultaneously minimizing the environmental impact of cropping systems (Searchinger, 2013).

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One of the most promising technologies to tackle these rising challenges consist in the use of plant biostimulants which include natural substances, other than fertilizers and pesticides, able to promote plant growth, yield, and to improve produce quality as well as resource use efficiency when applied to the crop in low quantities (Colla and Roupael, 2015; du Jardin, 2015). In the United States, the Association of American Plant Food Control Officials (www.aapfco.org) defined plant biostimulants as beneficial substances, which means “any substance or compound other than primary (i.e., N, P, K), secondary (i.e., Ca, Mg, S), and microplant nutrients (i.e., Fe) that can demonstrate by scientific research to be beneficial to one or more plant species when applied exogenously.”

Beneficial substances such as seaweed extracts (SWEs), particularly the brown algae

(Phaeophyceae), protein hydrolysates (PHs) and plant extracts (PEs) have been shown to play multiple roles as biostimulants through the regulation and modification of the primary and secondary metabolism in plants to enhance productivity and to minimize the impact of abiotic stress on crops (Calvo et al., 2014; Roupael et al., 2017c). Direct effects of biostimulant substances and compounds comprise the stimulation of enzyme activities involved in glycolysis, Krebs cycle, and nitrate assimilation as well as hormonal activities (Battacharyya et al., 2015; Colla et al., 2015b). The applications of these natural substances have been demonstrated to improve the nutrient uptake and nutrient use efficiency for both macroelements and microelements (Battacharyya et al., 2015; Colla et al., 2015b; Halpern et al., 2015). The higher nutrient use efficiency has been mostly associated with modifications in morphological root characteristics, such as root length and density, number of root hairs and their length, and hence their surface area (du Jardin, 2015). The biostimulant-mediated positive effects on plant nutrition, photosynthesis, and secondary metabolism can enhance vegetable quality (Battacharyya et al., 2015; Colla et al., 2015b). Ertani et al. (2014) revealed an increase of carbohydrates, antioxidant activity, lycopene, phenolic content, ascorbic acid, and capsaicin of greenhouse chili pepper (*Capsicum chinense* L.) grown in pot culture in response to the foliar application of a PH- and PE-based biostimulant.

However, except for few cases, the response of vegetable crops to biostimulant application was mainly investigated at the cell/tissue level (bioassays) and seedling stage under hydroponic or substrate culture (Colla et al., 2013, 2014; Lucini et al., 2015; Matsumiya and Kubo, 2011; Roupael et al., 2017a, 2017c). No published data are available concerning the effect of plant-derived biostimulants and SWE on yield, and especially fruit quality, of an important vegetable crop such as fresh-market tomato grown under soil conditions. Understanding how biostimulants can modulate the yield performance and phytochemical quality of greenhouse tomato under soil culture is a pressing need among growers, extension specialists, and scientists.

Taking this framework into consideration, the aims of the current study were 1) to comparably evaluate the effects of three biostimulants (PH, SWE, and PE) on yield performance characteristics, fruit morphometric characteristics, and fruit physicochemical composition, 2) to assess the associations between nutritive traits, and 3) to depict the economic profitability (i.e., additional net income) of biostimulant applications.

Materials and Methods

Experimental site, growth conditions, and plant material. The experiment was carried out in summer-autumn 2016, in a greenhouse covered by ethyl vinyl acetate (EVA) 0.25 mm

plastic film located at Terracina, Latina Province, central Italy (lat. 41°17'30"N, long. 13°14'36"E; altitude 22 m above sea level). The mean air temperature and relative humidity inside the greenhouse were 24.6 °C and 56%, respectively. The soil was sandy (89% sand, 4% silt, and 7% clay), with a bulk density of 1.1 g·cm⁻³, pH of 7.3, electrical conductivity of 1.4 dS·m⁻¹, organic matter of 0.2% (w/w), total N at 0.01%, available P at 6 mg·kg⁻¹, and exchangeable K at 15 mg·kg⁻¹.

The crop plant tested for the present study was tomato (*Solanum lycopersicum* L.) cv. Sir Elyan (Vilmorin INC, Tucson, AZ). The cultivar is an F1 hybrid widely cultivated under greenhouse conditions in Italy because of its good production potential, fruit setting, and steady fruit shape. 'Sir Elyan' is characterized as a large plum shaped fruit and is less sensitive to blossom end rot. The tomato seedlings were transplanted on 1 July at the three-true-leaf stage. 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 1.2 m, resulting in a plant density of 3 plants/m².

Biostimulant applications, experimental design, and crop management. Four treatments were derived from four biostimulant application treatments (control, PE, PH, or SWE). The treatments were arranged in a randomized complete-block design with three replicates per treatment, amounting to a total of 12 experimental unit plots with 50 plants each ($n = 600$ plants). The commercial PE 'Auxym', plant-derived PH 'Trainer', and SWE 'Kelpak' available in European and U.S. markets were used in the current study.

The PE 'Auxym' (Italpollina USA Inc., Anderson, IN) is a commercial plant biostimulant produced through water extraction and fermentation of tropical plant biomass. It contains phytohormones (mostly auxins 1.81 mg·kg⁻¹, cytokinins 0.29 mg·kg⁻¹ and auxin:cytokinin ratio 6:1), amino acids (51.9 g·kg⁻¹), vitamins (mostly niacin 3.3 g·kg⁻¹, vitamin C 1.0 g·kg⁻¹, vitamin E 0.4 g·kg⁻¹, thiamine 0.3 g·kg⁻¹, pyridoxine 0.3 g·kg⁻¹ and riboflavin 0.2 g·kg⁻¹), and other minor organic compounds (phytochelatins and enzymes); the elemental composition of 'Auxym' is as follows: N 8.3 g·kg⁻¹, P 4.0 g·kg⁻¹, K 25 g·kg⁻¹, Ca 0.9 g·kg⁻¹, Mg 1.2 g·kg⁻¹, Fe 6.6 g·kg⁻¹, Mn 6.4 g·kg⁻¹, B 4.4 g·kg⁻¹, Zn 0.4 g·kg⁻¹, and Cu 0.2 g·kg⁻¹. The legume-derived protein hydrolysate 'Trainer' (Italpollina USA Inc.) was also used in the current study. 'Trainer' is a commercial biostimulant obtained through enzymatic hydrolysis of proteins from legume seeds. It contains mostly soluble peptides (290 g·kg⁻¹) and to a lesser extent free amino acids (10 g·kg⁻¹) and soluble carbohydrates (90 g·kg⁻¹); the elemental composition of 'Trainer' is as follows: N 50.0 g·kg⁻¹, P 0.9 g·kg⁻¹, K 41.1 g·kg⁻¹, Ca 10.9 g·kg⁻¹, Mg 0.5 g·kg⁻¹, Fe 24.4 mg·kg⁻¹, Mn 1.0 mg·kg⁻¹, B 5.5 mg·kg⁻¹, Zn 9.6 mg·kg⁻¹, and Cu 1.2 mg·kg⁻¹. 'Trainer' also contains a soluble peptide called "root hair promoting peptide" having an auxin-like activity (Colla et al., 2014). Moreover, the third commercial

biostimulant used in the current study was the SWE 'Kelpak' [Kelp Products (Pty) Ltd, Cape Town, South Africa]. 'Kelpak' is produced from the brown seaweed, *Ecklonia maxima* (Osbeck) Papenfuss, which is harvested from the west coast of South Africa. 'Kelpak' is obtained through a process called "cold cell burst" (i.e., high pressure and low temperatures). It especially contains phytohormones (mostly auxins 11 mg·kg⁻¹, cytokinins 0.03 mg·kg⁻¹ and auxin:cytokinin ratio 367:1), carbohydrates (16.9 g·kg⁻¹), amino acids (2.5 g·kg⁻¹), vitamin B1 (0.9 mg·kg⁻¹), B2 (0.1 mg·kg⁻¹), C (20 mg·kg⁻¹), and E (0.7 mg·kg⁻¹); the elemental composition of 'Kelpak' is as follows: N 3.6 g·kg⁻¹, P 8.2 g·kg⁻¹, K 7.2 g·kg⁻¹, Ca 0.8 g·kg⁻¹, Mg 0.2 g·kg⁻¹, Fe 13.6 mg·kg⁻¹, Mn 8.4 mg·kg⁻¹, B 0.24 mg·kg⁻¹, Zn 4.2 mg·kg⁻¹, and Cu 0.2 mg·kg⁻¹. The previously mentioned biostimulants were selected as the most representative nonanimal-derived biostimulant categories (i.e., PEs, PHs, and SWEs) used in Italy and other European countries.

The treated plants were uniformly sprayed four times during the growing cycle at 10-day intervals with a solution containing 1 mL·L⁻¹ of PE 'Auxym', 3 mL·L⁻¹ of SWE 'Kelpak', and 3 mL·L⁻¹ of PH 'Trainer' using a 25-L stainless steel sprayer. The relative doses of the three biostimulants were used based on the manufacturer recommendations as well as based on previous results (Colla et al., 2014; Rouphael et al., 2017c; Sánchez, 2002). Foliar applications were initiated at the fruit set of the first truss (1 Aug.; 30 days after transplanting). The volumes of the solutions used during the four foliar applications were 1000, 1200, 1800, and 2000 L·ha⁻¹, respectively. Furthermore, the total amount of commercial biostimulants applied during the growing cycle were 6 L·ha⁻¹ of 'Auxym' and 18 L·ha⁻¹ of 'Kelpak' and 'Trainer'. The nutrient supplied by the biostimulant applications was negligible (less than 3% of the total nutrient applied) compared with the total nutrient supplied through fertigation.

The nutrient solution was delivered through fertigation with a composition of (in mmol·L⁻¹) 12.0 N-NO₃⁻, 1.8 S, 1.0 P, 7.0 K, 3.0 Ca, 1.8 Mg, and 3.5 N-NH₄⁺ 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 EC of 2.0 dS·m⁻¹ and a pH of 6.5. Drip lines, with in-line emitters located 0.30 m apart and an emitter flow of 2.4 L·h⁻¹, were placed 5 cm away from the tomato plants. 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 only water until the end of the day. This strategy was used to avoid a buildup of salts in the rhizosphere.

Crop was pruned at the end of August at the sixth truss stage. Pollination was facilitated by bumble bees. During the growing season, pests and pathogens were controlled by foliar treatments using the following pesticides: Altacor (active ingredient chlorantraniliprole), Affirm (active ingredient emamectin), Cabrio duo (active ingredient

pyraclostrobin + dimetomorf), Folicur (active ingredient tebuconazol), Laser (active ingredient spinosad), and Vertimec (active ingredient abamectin).

Yield assessment and SPAD index. Ripe fruit harvest was started on 2 Sept. and continued until the end of the experiment. The number of fruits, mean fruit mass, early (fruits harvested during the first week) and total yields were recorded on 20 plants located at the central part of each plot. Tomato fruits that were deformed or badly misshapen or rotten were considered unmarketable. The Soil Plant Analysis Development (SPAD) index was measured on fully expanded leaves by means of a portable chlorophyll meter SPAD-502 (Konica-Minolta corporation, Ltd., Osaka, Japan). Thirty leaves were randomly measured on 18 Sept. and averaged to a single SPAD value for each replicate. The experiment was ended on 8 Oct.

Fruit quality assessment. Immediately after harvest, the fruits of the fourth truss were analyzed for fruit quality parameters. Fruit shape index, defined as the ratio of width to length, was measured. Tomato fruit was excised and homogenized under low speed to prevent foaming. Part of the homogenate was filtered through the double cheesecloth and the total soluble solids (TSS) at 20 °C of the filtered juice was measured on a digital Atago N1 refractometer (Atago Co. Ltd., Tokyo, Japan). Part of the homogenate was transferred to falcon tubes, instantly dip-frozen in liquid nitrogen, and then stored at -80 °C for further phytochemical analyses. Fruit juice pH was also measured with a pH meter (HI-9023; Hanna Instruments, Padova, Italy). The fruits' dry matter content was determined as a percentage of fresh weight (FW) after fruit desiccation to constant weight in a forced-air oven at 80 °C for 72 h.

Lycopene content was determined according to Sadler et al. (1990). Briefly, 5 g of homogenized sample was extracted by 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 FW was obtained by measuring the absorbance of the lycopene hexane solution at 472 nm. Pure lycopene (Sigma-Aldrich, St. Louis, MO) was used for the preparation of calibration curves.

The hydrophilic fraction (HAA) from freeze-dried fruits (200 mg) was extracted with distilled water and its antioxidant activity was measured with the N,N-dimethyl-p-phenylenediamine 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 hydrophilic and lipophilic antioxidant activities were determined by using an ultraviolet-visible spectrophotometry. The absorbance of the solutions was measured at 505 and 734 nm, respectively. The 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 (DW), respectively.

The total ascorbic acid defined as ascorbic acid and dehydroascorbate acid was also assessed by a spectrophotometric detection as described by Kampfenkel et al. (1995). The absorbance of the solution was measured at 525 nm, and data were expressed as mg ascorbic acid on 100 g FW.

The total phenolic content in methanolic extracts was determined using the Folin–Ciocalteu 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–Ciocalteu's reagent (Sigma-Aldrich Inc) and 400 µL of 7.5% sodium carbonate/water (w/v). Absorption was measured after 30 min at 765 nm by using an ultraviolet-visible spectrophotometer, and the result was expressed as mg gallic acid (Sigma-Aldrich Inc) per 100 g DW.

Mineral analysis. Dried tomato fruit tissues were ground in a Wiley Mill to pass through an 841 microns screen and then portions of the dried tissues were used for mineral analysis. Total N concentration in fruit tissue was determined by Kjeldahl method after mineralization with sulphuric acid in the presence of potassium sulfate and low concentration of copper catalyst (Bremner, 1965). Macronutrients (NO₃, S, P, K, Ca, Mg) and sodium were extracted from 0.25 g samples with deionized water at 80 °C in a shaking water bath for 10 min. The resulting solution was filtered, diluted, and analyzed by ion chromatography (ICS-3000; Dionex, Sunnyvale, CA). A conductivity detector with IonPac CG12A guard column and IonPac CS12A analytical column was used for the analysis of K, Ca, Mg, and Na, whereas for NO₃, S, and P an IonPac AG11-HC

guard column and IonPac AS11-HC analytical column were used (Dionex).

Partial budget analysis. Partial budget analysis was performed to evaluate the economic advantage that may accrue to growers applying the biostimulants. The economic procedure used in the present work has been described by Djidonou et al. (2013). For each biostimulant treatment, the variable costs (added costs) and benefit (added gross return) of applying the biostimulant relative to the untreated control were calculated. The added net return incurred by each biostimulant was also calculated as the difference between added gross return and added variable costs.

Statistical analysis. An analysis of variance of the data was carried out using the SPSS 10 software package for Windows 2001. Quality traits were subjected to principal component analysis (PCA) to explore relationships among variables and treatments and to determine which quality traits were the most effective in discriminating between biostimulant application treatments. The PCA outputs include variable loading to each selected component and treatment component scores.

Results and Discussion

Yield, yield components, and SPAD index. The mean SPAD index in leaves was 51 (data not shown). The unmarketable yield was also unaffected by biostimulant treatments (data not shown; average 1.2 t·ha⁻¹). The foliar application of PE, SWE, and PH improved the early yield of fresh tomato by 22.0%, 14.1%, and 30.0%, respectively, in comparison with untreated plants, with no significant difference between the biostimulant treatments (Table 1). Similarly, to early yield,

the total marketable yield in biostimulant-treated plants was significantly higher than those in untreated plants (Table 1). The highest marketable yield was observed on plants treated with tropical PE, followed by both SWE and legume-derived PH, whereas the untreated plants exhibited the lowest fresh tomato production (Table 1). The higher marketable production observed on plants treated with tropical PE and to a lesser extent with SWE and PH, in comparison with untreated plants, was due to an increase in the number of fruits per plant, not in the fruit mean mass (Table 1).

The increase in early and total marketable fruit yields has been reported for other crops in limited experimental studies testing the action of biostimulants (i.e., PH and SWE) under open-field and greenhouse conditions (Ali et al., 2016; Halpern et al., 2015; Roupael et al., 2017c). These plant growth-stimulation effects (i.e., higher crop productivity) appear to be distinct from the nutritional effect of additional fertilizer (Ali et al., 2016; Calvo et al., 2014). Our results on SWE were consistent with the findings of Ali et al. (2016), who observed that the foliar application of *Ascophyllum nodosum* SWE (0.5%) enhanced the fruit yield of potted tomato (+54%) compared with the control. The higher performance was associated with the presence of polysaccharides in *Ascophyllum nodosum* SWE as sugars are known to improve plant productivity stimulating endogenous hormone homeostasis (Rolland et al., 2002). Furthermore, the high auxin:cytokinin ratio in SWE 'Kelpak' may also have contributed to the increase of fruit set especially during the first part of the growing cycle (August) when the high day-time temperature (up to 36 °C) may have impaired fertilization through a reduction of pollen viability (Singh and Shono, 2012).

Table 1. The effect of biostimulant treatments on early and total marketable yield, marketable fruit mean mass, and number of tomato fruits.

Biostimulant	Early yield (t·ha ⁻¹)	Marketable yield (t·ha ⁻¹)	Marketable fruit	
			Mean mass (g/fruit)	Number (no./plant)
Control	15.0 b	83.0 c	95.7	28.9 c
Tropical plant extract	18.3 a (22.0) ²	92.7 a (11.7)	95.5	32.4 a
Seaweed extract	17.1 a (14.1)	88.5 b (6.6)	96.6	30.5 b
Legume-derived protein hydrolysate	19.5 a (30.0)	88.8 b (7.0)	96.5	30.8 b
Significance	***	**	NS	**

²The percentage of increase in biostimulant treatments with respect to the control is reported in parenthesis.

NS, **, ***Nonsignificant or significant at $P < 0.01$ or 0.001 , respectively. Different letters within each column indicate significant differences according to Duncan's multiple range test $P < 0.05$.

Table 2. The effect of biostimulant treatments on dry matter percentage, total soluble solids, juice pH, lipophilic and hydrophilic antioxidant activities, and total phenols and total ascorbic acid of tomato fruits.

Biostimulant	Dry matter (%)	Total soluble solids (°Brix)	pH	Antioxidant capacity		Total phenols (mg gallic acid eq./100 g DW)	Total ascorbic acid (mg/100 g FW)
				Lipophilic (mmol Trolox eq./100 g DW)	Hydrophilic (mmol ascorbic acid eq./100 g DW)		
Control	5.87	4.00 b	4.45	1.30	2.36 a	13.7	122.1
Tropical plant extract	5.22	3.80 b	4.49	1.65	2.01 ab	9.9	117.8
Seaweed extract	5.24	3.93 b	4.49	1.75	2.04 ab	15.0	168.6
Legume-derived protein hydrolysate	5.77	4.57 a	4.53	1.72	1.78 b	14.6	137.1
Significance	NS	**	NS	NS	*	NS	NS

NS, *, **Nonsignificant or significant at $P < 0.05$ or 0.01 , respectively. Different letters within each column indicate significant differences according to Duncan's multiple range test $P < 0.05$.

Foliar applications of synthetic auxins such as naphthaleneacetic acid have shown to stimulate parthenocarpic fruit set and to increase tomato fruit yield (Alam and Khan, 2002; de Jong et al., 2009). Similarly to SWE, the foliar application of legume-derived PH increased the marketable yield by 7%, compared with untreated plants. Koukounararas et al. (2013) showed that the root or foliar application of a PH-based commercial product Amino 16 containing 11.3% L-amino acids enhanced yield and yield components of greenhouse tomato irrespective of the fertilization rate. Colla et al. (2014) demonstrated that the legume-derived PH 'Trainer', used in the current experiment, elicited an auxin-like activity and a weak gibberellin-like activity in a set of bioassays. It has been reported that beyond auxins, the gibberellins also play a major role in the onset of tomato fruit development by controlling together the expression of the genes regulating cell division and cell expansion in fruits (de Jong et al., 2009). However, the most significant improvement in marketable yield was observed when the application of PE was adopted. A presumed mechanism involved in the stimulation caused by the PE application might be the presence of phytohormones (e.g., auxins) and signaling compounds (e.g., amino acids, vitamins, phytochelutins). The phytohormones and signaling compounds, typical components of 'Auxym', may have improved the photosynthetic activity and fruit set leading to a better yield. Another possible mechanism involved in the significant biostimulant effect of PE could be related to the stimulation of root growth of treated plants, which may have improved water and nutrient uptake efficiency, leading to an overall increase in crop productivity.

Quality attributes of fresh tomato. No significant differences between the four biostimulant treatments were observed for the fruit shape index (average 0.62; data not shown), dry matter content (average 5.52), and juice pH (average 4.49) (Table 2). The nonsignificant effect of biostimulant applications on shape index could be expected because fruit shape constitutes a trait predominantly governed by the genotype and little affected by environmental or cultural factors (i.e., biostimulants) (Kyriacou et al., 2017). Concerning the TSS content, an important trait for consumer satisfaction reflecting sweetness (Kader, 2002), our results indicated that the highest value was observed when tomato plants were treated with legume-derived PH (Table 2). These data are consistent with a previous study in which foliar treatments with two biostimulants, one derived from alfalfa plants and the other obtained from red grape, resulted in an increase of glucose and sucrose of potted chili pepper (Ertani et al., 2014).

In the current study, the LAA and HAA of fresh tomato ranged from 1.30 to 1.75 mmol Trolox eq./100 g DW and from 1.78 to 2.36 mmol ascorbic acid eq./100 g DW, with the lowest levels of HAA recorded with legume-derived PH treatment (Table 2).

Fig. 1. The effect of biostimulant treatments on lycopene (A) and nitrate (B) contents of tomato fruits. The values are means of three replicates. Vertical bars indicate $\pm$ SE of means. Different letters indicate significant differences according to Duncan's test ($P < 0.05$).

Table 3. Effect of biostimulant treatments on mineral composition of tomato fruits.

Biostimulant	Mineral elements (g·kg ⁻¹ DW)						
	N	P	S	K	Ca	Mg	Na
Control	19.1	6.6	19.0	28.6 b	4.3 b	5.7 ab	0.63
Tropical plant extract	20.5	5.7	18.1	30.0 ab	4.1 b	5.4 ab	0.65
Seaweed extract	21.5	6.4	20.6	28.8 b	4.8 a	4.6 b	0.60
Legume-derived protein hydrolysate	20.4	6.5	20.4	32.4 a	4.5 ab	6.2 a	0.73
Significance	NS	NS	NS	**	*	*	NS

NS, *, **Nonsignificant or significant at $P < 0.05$ or 0.01 , respectively. Different letters within each column indicate significant differences according to Duncan's multiple range test $P < 0.05$.

Neither the total phenols (13.1 mg gallic acid/100 g DW) nor the total ascorbic acid (136.4 mg/100 g DW) were influenced by

biostimulant treatments (Table 2). Contrarily to total phenols and ascorbic acid contents, lycopene was highly ($P < 0.01$) affected by

Fig. 2. Principal component loading plot (A) and scores of principal component analysis (B) of tomato fruits as a function of biostimulant treatments (control, tropical plant extract, seaweed extract, and legume-derived protein hydrolysate). AA, total ascorbic acid; DM, fruit dry matter percentage; HAA, Hydrophilic antioxidant capacity; LAA, Lipophilic antioxidant capacity; TSS, total soluble solids.

Table 4. Added returns and variable costs resulted by biostimulant applications under greenhouse conditions compared with untreated control.

Biostimulant	Added gross return ^z (\$/ha)	Added variable costs (\$/ha)				Added net return (\$/ha)
		Biostimulant product ^y	Foliar spraying ^x	Fruit harvest ^w	Total	
Tropical plant extract	5,820	144	400	2,134	2,678	3,142
Seaweed extract	3,300	270	400	1,210	1,880	1,420
Legume-derived protein hydrolysate	3,480	272	400	1,276	1,948	1,532

^zThe tomato selling (shipping point) prices of 600 \$/t was used in calculating the gross returns of biostimulant-treated tomato production.

^yCosts of biostimulants were provided by suppliers (Auxym = 24.0 \$/L; Kelpak = 15.0 \$/L; Trainer = 15.1 \$/L).

^xCosts of foliar spraying were calculated based on the information provided by local agricultural contractors.

^w220 \$/t for labor used to harvest tomatoes as reported by Testa et al. (2014).

biostimulant treatments (Fig. 1A). The lycopene content was the highest by 125% when the foliar application of the commercial legume-derived PH ‘Trainer’ was delivered (Fig. 1A). The beneficial effects of legume-derived PH on phytochemical compounds (i.e., lycopene) could be related to the activation of molecular and physiological mechanisms (Ertani et al., 2011, 2014). The production and accumulation of lycopene with PH application could be considered as an *extra value* to support human health (Erba et al., 2013).

Concerning the undesirable components, such as nitrates, the nitrate content was significantly influenced ($P < 0.01$) by the biostimulant treatments with the lowest values recorded on plants treated with tropical PE (Fig. 1B). In general, nitrate accumulating vegetables belong to the families of *Brassicaceae*, *Chenopodiaceae*, and *Asteraceae*, whereas fruit vegetables, such as tomato, are characterized by very low ($<200 \text{ mg}\cdot\text{kg}^{-1} \text{ FW}$) nitrate accumulation (Santamaria, 2006). This was the case in the current experiment because the concentrations of nitrates in fresh tomato ranged between 67.2 and 120.1 $\text{mg}\cdot\text{kg}^{-1} \text{ FW}$. The ability of tropical PE also containing amino acids to minimize the accumulation of nitrates could be associated with the regulation of several metabolic pathways of plant N metabolism, in particular, nitrite and nitrate reductase, as well as glutamate synthase and glutamine synthetase activities (Calvo et al., 2014; Colla et al., 2015b). In line with our results, several studies carried out on barley, maize, soybean, and wheat (Liu and Lee, 2012) demonstrated that exogenous amino acids and amides could downregulate nitrate uptake.

Mineral composition. For a balanced and good nutrition in human diet, fruits and vegetables should be consumed to fulfil all nutritional needs in terms of minerals and vitamins. Levander (1990) reported that vegetables contribute normally by 11%, 35%, 7%, and 24% to the human dietary intake of P, K, Ca, and Mg, respectively.

Among the major elements studied, K was the main mineral constituent of tomato fruit (Table 3). Concerning the influence of biostimulant treatments on mineral profiling, a significant variation was observed for K, Ca, and Mg, whereas no significant differences among biostimulant applications were found for N ($20.4 \text{ g}\cdot\text{kg}^{-1} \text{ DW}$), P ($6.3 \text{ g}\cdot\text{kg}^{-1} \text{ DW}$), and Na ($0.6 \text{ g}\cdot\text{kg}^{-1} \text{ DW}$) concentrations (Table 3). Legume-derived PH increased K and Mg concentrations, thereby increasing the nutritional value of the fruit. Furthermore, the application of *E. maxima* extract and to a lesser degree PH enhanced the Ca concentration in the fruit tissue (Table 3). Increased mineral concentration in the fruit may be associated with the presence of bioactive compounds (e.g., amino acids, peptides, and carbohydrates) in biostimulants which may have increased sink strength influencing the movement of substrates, including minerals, within the

plant (Calvo et al., 2014). Moreover, Kossak and Dyki (2008) showed more numerous and larger xylem cells and phloem vascular bundles in the stems of tomato plants treated with biostimulants compared with the untreated plants. This phenomenon can contribute to a more effective translocation of minerals and photosynthates to the sinks. The increased mineral concentration in fruit of treated plants may also be due to a greater absorption of minerals through a stimulation of root growth and the activity of nutrient transporters in cell membranes (Billard et al., 2014; Colla et al., 2014).

Lastly, the present data showed that higher lycopene content found in PH-treated fruits could be related to K concentration in tomato. The positive effect of K on lycopene content has been reported previously by Fanasca et al. (2006a, 2006b). Bramley (2002) demonstrated that K can be involved in the process of carotenoid biosynthesis by its action on the activity of enzymes (pyruvate kinase and phosphofructokinase) that regulate carbohydrate metabolism.

Principal component analysis. The first three principal components (PCs) were associated with eigenvalues >1. PC1 which explained 42.2% of the variance was positively correlated with lycopene, TSS, juice pH, K, S, and Na and negatively correlated with HAA (data not shown). Moreover, PC2 and PC3, which explained 33.3% and 24.5% of the variance, respectively, were positively and strongly correlated with total AA (ascorbic acid), LAA, N, Ca (for PC2) and total phenols, nitrate, and P (for PC3) (data not shown). The loading plot in Fig. 2A illustrates the relations among quality traits. For instance, variation in K was most closely aligned to that of TSS content, and variation in LAA content was more strongly correlated with Ca (i.e., narrower angle between the corresponding vectors) rather than total AA content. Similarly, K was more strongly correlated with lycopene content than with total phenols and HAA.

Recent scientific articles have demonstrated the effectiveness of PCA plotting in genetic classification and preharvest and postharvest quality studies (Colonna et al., 2016; Kyriacou et al., 2016; Roupael et al., 2016, 2017b). This was the case in the current experiment because PCA scores have introduced concerted information on the quality attributes in relation to biostimulant treatments. The two PCs score plot separates and categorizes biostimulant treatments into four groups (Fig. 2B). The upper right quadrant in the positive side of PC1 included the seaweed extract treatment. Tomato plants treated with SWE were mostly characterized by high total ascorbic acid, N and Ca contents (Fig. 2B). The cluster in the lower right quadrant (i.e., the negative side of the PC1) represents tomato fruits treated with legume-derived PH, characterized by high TSS, K, Mg, and lycopene contents (Fig. 2B). The lower left quadrants (negative side of PC1), which clustered two

biostimulant treatments, tropical PE and control, depicted the treatments of the lowest quality traits with the exception of HAA. The PCA conducted in the present study may provide the basis for a more in-depth approach to elucidate the effects of biostimulant treatments on the quality traits of greenhouse fresh tomato.

Partial budget analyses of biostimulant tomato production. In the current experiment, the added marketable yields due to biostimulant applications compared with the control were 9.7, 5.5, and 5.8 t·ha⁻¹ for tropical PE, SWE, and legume-derived PH treatments (data not shown). Consequently, the positive effects associated with biostimulant-treated plants involved the added gross returns on fresh tomato values, which ranged from 3300 to 5820 \$/ha with the highest added returns recorded in plants treated with PE (Table 4). The total added variable costs were biostimulant-dependent and varied from 1880 to 2678 \$/ha with the highest negative effects recorded with tropical PE applications (Table 4). Among the total variable costs, fruit harvest was the main cost item (64% to 80% of total costs), followed by the foliar spraying (15% to 21% of total costs) and finally the cost of the plant biostimulants (5% to 14% of total costs) (Table 4). After accounting for plant biostimulant, foliar spraying, and fruit harvest costs, the net returns of biostimulant-treated to untreated plants were 3142, 1420, and 1532 \$/ha for tomato plants treated with PE, SWE, and PH, respectively (Table 4). These results indicated that the increased crop value was associated with the significant improvement of total marketable production, in particular with PE, making it more profitable than untreated fresh tomato production.

Conclusions

In conclusion, our findings indicated that biostimulant-treated plants enhanced yield and yield components of greenhouse tomato compared with untreated plants especially when the foliar application of the commercial tropical PE 'Auxym' was delivered. Qualitative differences in fruit quality traits were identified between control-, PE-, SWE-, and PH-treated tomato plants. The quality attributes of fresh tomato (TSS, lycopene, K, and Mg contents) were improved by the applications of legume-derived PH 'Trainer'. Growing biostimulant-treated tomatoes incurred higher production costs; however, the yield increase with the use of biostimulants led to higher gross returns that ultimately improved the net returns as compared with the cultivation of untreated plants.

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