



Research Paper

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



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ARTICLE INFO

Keywords:

Antioxidant activity
Lycopene
Mineral composition
Plant biostimulants
Solanum lycopersicum L.
Sustainable horticulture

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 CO₂, 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 of 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 CO₂. 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.

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 Vandenkoornhuysse, 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 microelement plant nutrients (i.e., Fe, Mn, Zn, Cu), that can be demonstrated 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 well-known 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 on the market as liquid products, soluble powder or in 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-

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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 (Roupael et al., 2017a). Direct effects of PHs on plants include fostering the activity of key enzymes (i.e., aspartate 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 elicit 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 CO₂ 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 °C 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 and 40 g (small size) with 15–20 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 around the world. 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 indeterminate 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 m² 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, carbohydrates (90 g kg⁻¹) and free amino acids (Colla et al., 2015; Lucini et al., 2015).

The treated plants were uniformly sprayed nine times during the growing cycle at 10-day intervals using a 16-L stainless steel sprayer 'Vibi Sprayer' (Volpi, Piadena, Italy). Foliar application was initiated 15 days after transplanting (17 August). The two Trainer® concentrations were used on the basis of the manufacturer's recommendations. Crop was pruned at the 6th truss stage. Pollination was facilitated by bumble bees introduced by means of a 'Natupol' hive (Koppert Italia S.R.L., Bussolengo, Italy) upon full flowering of the first truss. During the growing season, pests (spider mites, leaf miners, 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-NO₃⁻, 1.5 S, 1.0 P, 6.0 K, 4.0 Ca, 2.0 Mg, 1.5 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 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 °C 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.

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