



Plant biostimulants: innovative tool for enhancing plant nutrition in organic farming

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Summary

Organic farming has generated significant interest among consumers and scientists owing to their healthier and safer characteristics to human health. However, nutrient (N and P) availability has been identified to be a major yield-limiting factor in many organic farming systems. Plant biostimulants which are defined as substances (humic acids, protein hydrolysates and seaweed extracts) and/or microbial inoculants (plant growth promoting rhizobacteria, arbuscular mycorrhizal fungi and *Trichoderma* spp.) applied to plants with the aim to enhance plant resilience and also to improve nutrient uptake and translocation. This review focuses on how the plant biostimulants application can overcome nutrient limitation typical of organic systems by improving nutrient availability, uptake and assimilation, and consequently reducing the gap between organic and conventional yields. The agronomical, physiological and molecular responses conferring tolerance to nutrient deficiency were also covered. The review concluded by proposing several research areas to understand the specific application of plant biostimulants to improve the yield of organic horticulture.

Keywords

arbuscular mycorrhizal fungi, humic acids, N-fixating bacteria, nutrient transporters, protein hydrolysates, P-solubilization, vegetables, yield gap

Introduction

Over the past two decades, interest in organically grown vegetables has been on the rise worldwide, as a result of growing interest of the consumers and scientists in healthy and safer products (Dorais and Alsanus, 2016). In fact, organic horticulture has increased worldwide by almost two-fold since 2008, accounting for 3.5 million ha of cultivated organic land in 2014, with more than 87 countries practicing organic agriculture (Willer and Lernoud, 2016). Furthermore, organic horticulture has been often reported as an environmentally-friendly production system able to produce food with minimal harm to ecosystems (i.e., water and soil conservation) as well as minimal use of off-farm inputs (Dorais, 2007). However, the major drawback of organic farming is the lower yield compared to conventional agriculture (Seufert et al., 2012; Dorais and Alsanus, 2015). Therefore, more land is needed to produce the same amount of food as conventional farming, leading to more deforestation, and

Significance of this study

What is already known on this subject?

- Plant biostimulants have been gaining interest in sustainable agriculture due to their stimulation effects able to improve nutrient use efficiency, quality of the product and abiotic stress tolerance.

What are the new findings?

- The aim of this review article was to highlight recent advances on the use of natural substances (humic acids, protein hydrolysates, seaweed extracts) and microbial inoculants (plant growth promoting rhizobacteria, arbuscular mycorrhizal fungi and *Trichoderma*), also called plant biostimulants, to overcome nutrient limitation in organic farming for an optimal plant growth.

What is the expected impact on horticulture?

- The overall impact of this review will be to set a scientific frame to identify how the plant biostimulants treatments (substances and/or microorganisms) have the potential to enhance plant resilience to nutrient limitation typical of organic farming, and consequently reducing the gap between organic and conventional yields.

consequently reducing the environmental benefits of organic practices (Trewavas, 2001).

Crop productivity reduction under organic regime has been recently addressed in two meta-analyses (Seufert et al., 2012; Ponisio et al., 2015). Based on these studies, organic yield was 20% lower than conventional ones (Ponisio et al., 2015), whereas the yield reduction reported by Seufert and co-workers ranged between 5 and 34%. The yield reduction observed in organic horticultural farming was mainly associated to a major biotic pressure (both seed-borne and shoot fungal and bacterial diseases; Van Bueren et al., 2011; Ponisio et al., 2015; Orsini et al., 2016) as well as nutrient limitation (de Ponti et al., 2012). Indeed, nutrient (i.e., N and P) availability has been found to be the main yield-limiting factor in many organic production systems (Clark et al., 1999; Berry et al., 2002). The release of plant available mineral N and P from organic fertilizers such as fish and meat meal, pelleted chicken manure, bat guano and seabird, bone, blood and crop residues (alfalfa and soybean) (Tuomisto et al., 2012) is often not synchronized with crop demand leading to mismatch between nutrients bioavailability, in particular nitrogen and plant uptake during the peak growing period (Pang and Letey, 2000; Zhao et al., 2009; Lester and Saffner,

2011). Moreover, nutrient availability (e.g., P, Fe, Mn, Zn, Cu) for plant uptake can be further reduced under adverse soil pH (alkali or acid) due to the formation insoluble complexes (e.g., tricalcium phosphate in alkaline soils or ferric phosphate/aluminum phosphate in acid soils). Therefore, there is an urgent need to bring new tools to increase nutrient availability, plant uptake and assimilation in order to close the gap between organic and conventional yields (Barbieri et al., 2015; Rouphael et al., 2015a; De Pascale et al., 2016).

A promising tool and sustainable approach would be the use of naturally derived plant biostimulants (PBs), which is gaining interest globally (Colla and Rouphael, 2015; Rouphael et al., 2017a). The actual global market is estimated around \$ 1 billion and has been projected to reach more than \$ 3 billion by 2020, with a yearly growth of 13% especially in EU countries (Calvo et al., 2014; Colla and Rouphael, 2015). As recently defined by Patrick du Jardin (2015), PBs correspond 'to any substance or microorganism applied to plants with the aim to enhance nutrition efficiency, abiotic stress tolerance and/or crop quality traits, regardless of its nutrients content'. The major plant biostimulants are: humic and fulvic acids, protein hydrolysates, seaweed extracts, silicon, chitosan, inorganic compounds, beneficial fungi (i.e., arbuscular mycorrhizal fungi; AMF and *Trichoderma* spp.) and plant growth-promoting bacteria (Canellas et al., 2015; Colla et al., 2015a; Rouphael et al., 2015b; Ruzzi and Aroca, 2015).

Several studies conducted on greenhouse and open-field vegetables suggest that applications of biostimulant substances can promote nutrient uptake and assimilation (Colla et al., 2015a). The increase of plant nutrient uptake has been often attributed to one or more of the following factors: an increase in soil enzymatic and microbial activities, modifications in root architecture as well as an enhancement in micronutrient mobility and solubility (Ertani et al., 2009; Colla et al., 2013, 2014; Lucini et al., 2015). In addition to biostimulant substances, the use of microbial inoculants in horticulture has been also on the rise during the last two decades. Microbial inoculants mainly include plant growth promoting rhizobacteria (PGPR) and endophytic fungi such as AMF and *Trichoderma* spp. (Calvo et al., 2014; Colla et al., 2015b; Rouphael et al., 2017b). These useful bacteria and fungi have the potential to contribute to sustainable plant growth promotion even under nutrient limitation typical of organic farming. The plant growth promotion resulting from better nutrient uptake induced by microbial based biostimulants has been associated to several mechanisms such as (1) supplying soil nitrogen (i.e., by biological N₂ fixation); (2) making soil nutrients more available to plant uptake (i.e., by solubilization of mineral phosphates and other nutrients through the production of small metal-binding molecules such as organic acids and siderophores, and the release of specific enzymes like phosphatases); (3) increasing plant access to soil nutrients (i.e., by increasing the volume of soil accessed by the root system) (Hayat et al., 2010; Calvo et al., 2014; Colla et al., 2015b; Rouphael et al., 2015b).

The aim of this review article was to highlight recent advances on the use of PBs to improve plant nutrition and their potential application in organic farming. The current review will focus on natural substances (humic acids, protein hydrolysates, seaweed extracts) and microbial inoculants (PGPR, AMF and *Trichoderma*) as a means to overcome nutrient limitation for an optimal plant growth through the improvement of nutrient availability (i.e., by supplying available nitrogen, solubilizing soil nutrients), uptake (i.e., by increasing the volume of soil accessed by the root system, enhancing the

activity of nutrient transporters in root cell membranes), and assimilation (i.e., by increasing the activity of nitrogen assimilation enzymes in leaves and roots), and consequently reducing the gap between organic and conventional yields.

Soil nutrient availability

Plants grown in organic farming are often exposed to nutrient deficiency resulting from low amounts of nutrients in the soil or to the poor solubility of nutrients in soil solution. Plant biostimulants can enhance the nutrient availability for plant uptake by increasing cation exchange capacity of soil (reduction of nutrient leaching especially in sandy soils), by supplying nitrogen to the crops, and/or by enhancing the solubility of nutrients in soil solution.

Humic substances (HS) are natural substances resulting from the chemical and biological transformations of dead cell materials as well as from microbial metabolism (du Jardin, 2012; Canellas et al., 2015). HS have been recognized as essential contributors on the physico-chemical properties of soils. Moreover, most biostimulant effects of HS refer to stimulation of root growth and improvement of plant nutrition resulting from the increase of soil nutrient availability (Canellas et al., 2015; du Jardin, 2012, 2015). HS act on soil nutrient availability by increasing cation exchange capacity and buffering (neutralize) soil pH (Canella et al., 2015; du Jardin, 2015). Another important positive effect of HS on soil nutrient availability for plant uptake is the formation of soluble HS complexes with micronutrients (i.e., iron). The trace element-humic complex has been often considered as a strategy to improve plant nutrition of micronutrients by preventing leaching and making micronutrients more available to plants (Chen et al., 2004; Garcia-Mina et al., 2004). Moreover, intact micronutrient cation-fulvic acid complexes appeared to be taken up by the plants; this is due to the low molecular size of fulvic acids, which easily reach the plasma-lemma and enter in the plant cells whereas the high molecular size fraction (humic acids) are not absorbed and can interact only with the cell wall (Nardi et al., 2002). It is also well established that application of HS stimulates plasma membrane H⁺-ATPase activity, thereby increasing H⁺ extrusion from roots and lowering root surface pH, and thus triggering soil nutrient availability for a better uptake and translocation (Canellas et al., 2015).

Besides the significant effects on soil fertility in particular the physico-chemical and biological activities, HS may have also significant influence on secondary metabolism and stress alleviation. For instance, Zeng et al. (2002) showed that the carboxylic and phenolic hydroxyl groups of HS are the principal binding sites for metals in particular the heavy ones; HS bind heavy metals and in that way plant uptake of heavy metals is reduced. In fact, Shahid et al. (2012) and Santos et al. (2014) demonstrated that humic and fulvic acids are able to mitigate Pb toxicity by complexing highly toxic free Pb²⁺ in solution, and limiting its uptake translocation in plants, and thus reducing the risk transfer of these Pb²⁺ into the food chain. However, other authors (Evangelou et al., 2004; Park et al., 2012) have demonstrated a stimulatory effect of humic acids on the accumulation of heavy metals including Pb²⁺. An explanation of these contradictory results could be associated to soil properties, in particular the soil pH. In fact, it has been demonstrated that heavy metals pollution in acidic soils (pH < 5.0) yielded a higher environmental risk compared to alkaline soils (Zhang et al., 2013). However, Pb²⁺ accumulation in plants may be strongly correlated to other factors, in particular the soil pH.

The effectiveness of HS to improve salinity tolerance was also reported by Türkmen et al. (2004) and Paksoy et al. (2010) on tomato and okra, respectively. The former authors showed that HS application can induce salt tolerance by increasing root growth, decreasing membrane damage as well as improving the chemical, physical and microbiological properties of soil.

Another important category of plant biostimulants are the protein hydrolysates (PH) defined as 'mixtures of polypeptides, oligopeptides and amino acids that are manufactured from protein sources using partial hydrolysis' (Schaafsma, 2009). They are basically available as soluble granular or powder as well as liquid extracts and may be applied as foliar sprays or dosed near the root system (Colla et al., 2015a). PH can improve soil respiration, microbial biomass and activity, since microorganisms can easily use amino acids and peptides as C and N source (Farrell et al., 2014). They also impact on plant nutrition by forming complexes and chelates between peptides/amino acids and soil micronutrients (i.e., Cu, Fe, Mn and Zn), therefore contributing to nutrients availability and acquisition by the root system (du Jardin, 2015; Colla et al., 2015a). Moreover, the ability of peptides/amino acids to form complexes and chelates with some macro- (i.e., K, Ca and Mg) and micronutrients (i.e., Cu, Fe, Mn and Zn) are currently used by some industries to develop fertilizers having high nutrient use efficiency. In general PGPR or plant growth promoting fungi such as arbuscular mycorrhiza fungi (AMF) and *Trichoderma* spp. may induce plant growth promotion by improving the availability of nutrients such as N, P and Fe (Dorais, 2007). A variety of symbionts such as *Azorhizobium*, *Allorhizobium*, *Bradyrhizobium*, *Mezorhizobium*, *Rhizobium*, and *Sinorhizobium* and non-symbiotic nitrogen-fixing bacteria such as *Azospirillum*, *Azotobacter*, *Bacillus* and *Klebsiella* sp. are now being used worldwide aiming to enhance the availability of different nutrients including N, P and micronutrients, thus plant productivity under both conventional and organic farming systems (Hayat et al., 2010; Miransari, 2011; Bhardwaj et al., 2014; Calvo et al., 2014). For instance, *Azospirillum* as well as *Rhizobium* spp., a non-symbiotic and symbiotic bacteria are able to fix N₂ (Arzanesh et al., 2010; Miransari, 2011). Moreover, these beneficial soil microbes can play an important role in recycling organic nutrients, since soil-nitrifying bacteria are able to mineralize organic nitrogen to nitrite and then to nitrate, which can be easily absorbed by the crops (Miransari, 2011). Among all the microbial inoculants, *Azospirillum* is the most studied (Calvo et al., 2014). Several studies conducted under field experiments showed the significant capacity of *Azospirillum* spp. to fix atmospheric nitrogen (Boddey et al., 1991; Fayez and Daw, 1987; De Salamone et al., 2001; Saubidet et al., 2000; Malik et al., 2002). Significant increases in nitrogen content by inoculation with *A. brasilense* and *A. lipoferum* (7–12%) and *A. diazotrophicus* (60–80%) have been reported in wheat and sugarcane, respectively (Bodey et al., 1991; Malik et al., 2002). Moreover, several *Bacillus* species (*Bacillus megaterium*, *B. circulans*, *B. subtilis*, *B. polymyxa*, *B. sircalmous*) have been described as effective phosphate solubilizers (Mohammadi, 2012). The mechanisms of PGPR-mediated improvement to crop productivity are not fully elucidated (Dey et al., 2004). Some PGPR function as a sink for 1-aminocyclopropane-1-carboxylate (ACC), the immediate precursor of ethylene in higher plants, by hydrolyzing it into α -ketobutyrate and ammonia, and in this way promote root growth by lowering indigenous ethylene levels in the micro-rhizo environment (Hayat et al., 2010). Many PGPR are also able

to produce plant hormones such as auxins, cytokinins, gibberellins, ethylene and abscisic acid (Hayat et al., 2010). It is well known that auxins can stimulate root growth improving nutrient and water uptake of plant while cytokinins promote mitotic cell division in the shoots and roots, and delay leaf senescence. Gibberellins affect a number of physiological processes such as fruit and flower formation, dormancy of vegetative organs as well as seed germination (Métraux, 1987). Moreover, abscisic acid plays a crucial role in adaptive responses to environmental stresses, such as drought and high salinity (controlling stomata closure), as well as in plant development, such as seed maturation and dormancy (Miyakawa et al., 2013).

Similar to nitrogen, several PGPR can enhance solubility of phosphates, resulting in an increased availability of phosphate in the soil that can be assimilated by the plant (Canbolat et al., 2006; Liao et al., 2008). Different bacterial strains belonging to the genera *Aspergillus*, *Bacillus*, *Flavobacterium*, *Micrococcus* and *Pseudomonas* have been reported to be active in the solubilization of inorganic phosphate compounds (i.e., dicalcium and tricalcium phosphate and rock phosphate) (Pindi and Satyanarayana, 2012; Rodriguez and Faraga, 1999; Rodriguez et al., 2006). The two most demonstrated mechanisms responsible of the solubilization process are the production of organic acids and the production of phosphatase (Goldstein, 1995; Rodriguez et al., 2006). In addition to P, some PGPR strains *B. mucilaginosus* and *B. megaterium* were able to improve the availability of K and also to release K from its immobile forms in the soil (Han and Lee, 2005). Furthermore, through producing siderophores, PGPR as well as *Trichoderma atroviride* can enhance iron solubility and hence uptake and translocation by plant (Lugtenberg and Kamilova, 2009; Colla et al., 2015b).

Similarly to PGPR, AMF which are defined as beneficial associations between soil fungi and plant roots (Rouphael et al., 2010, 2015c, 2017b) can also enhance P availability under nutrient deficiency/availability typical of organic farming systems. The basis of the soil P availability improvement has been associated to the developed network of the external hyphae capable of extending the surface area for nutrient uptake and to the production of phosphatases and/or excretions of organic substances able to solubilize P (Marschner, 1998; Rouphael et al., 2015b). Moreover, mycorrhizal fungi can indirectly increase nutrient availability in the soil through the improvement of soil aggregate stability (resulting from the soil particle binding agent 'glomalin' released by AMF) which increases root growth and activity (Wu et al., 2014), through the increase of organic matter content in soil enhancing retention of cations such Ca²⁺ and Mg²⁺, and through the improvement of nitrification processes (Morrisson et al., 2017).

Plant nutrient uptake

Plant nutrient uptake depends on a number of factors, including plant species, environmental conditions, and microorganisms associated with plant roots. Root growth and function play a fundamental role in nutrient uptake especially in organic farming where nutrients are often available in soil solution at relatively low concentrations. Therefore, an extensive root system is a pre-requisite to guarantee a sufficient absorption of nutrients to meet crop demand in organic farming. Several studies have shown that plant biostimulants like HS, PHs and seaweed extracts (SWE) have the potential to boost the root growth and development allowing a better soil exploration and resource acquisition (nutrients and

water) by plant roots. It has been supposed that the presence of auxins in many PBs is most likely responsible for root stimulation. However, Wally et al. (2013) pointed out that the concentration of auxins in many PBs applied at the recommended rate, is generally well below the threshold for inducing root stimulation. Therefore, biostimulant effect on root initiation and growth has been often attributed to an interaction of biostimulant-derived organic molecules with cell membrane-localized receptors triggering a signal transduction pathway via modulation of the intracellular levels of the hormones (Battacharyya et al., 2015). Scaglia et al. (2016), studying the organic molecules responsible for auxin-like activity of humic acids, were able to identify several small organic compounds such as amino acids, carboxylic linear acids and aromatic carboxylic acid able to elicit auxin-like responses in plant like enhancement of root growth. Moreover, Zandonadi et al. (2010) reported that HS stimulated the expression of the early auxin-responsive genes IAA5 and IAA19 and induced plasma membrane H⁺-ATPase synthesis, activity and expression promoting lateral root induction and root hair growth. However, crop response to HS application can vary substantially (Hartz and Bottoms, 2010; Cannellas et al., 2015). Rose et al. (2014), using a random-effects meta-analysis model, estimated a root dry weight increases of 21% in response to HS application with a large variation in plant response depending on the source of the HS, the rate of HS application, and to a lesser extent, on the plant type and growing conditions. HS extracted from compost or vermicompost appeared to be more effective than those obtained from brown coal (Cannellas et al., 2015). Moreover, HS application rate affected nonlinearly the growth response of crop (Atiyeh et al., 2002). These findings suggest that specific dose-response curves are needed for optimizing the rate of HS in different cropping systems and environmental conditions. Crop response to humic acid application is usually greater in monocots than dicots, at early stages of plant growth and under low fertility conditions. All these factors should be taken into consideration for making successful HS applications in organic farming.

PHs have been also used to stimulate root growth in several crops like tomato, lettuce, and corn (Colla et al., 2013, 2014). Even if low levels of auxins have been found in plant derived-PH, root stimulation activity of PH has been often attributed to peptides and amino acids. It is well known that peptides play an important role as signaling molecules in regulating plant growth and development (hormone-like activity; Matsubayashi and Sakagami, 2006). These peptides are secreted by the cells and recognized by specific membrane receptors (e.g., receptor protein kinases) of surrounding cells allowing a cell-to-cell communication. Bioactive peptides have been also identified in plant-derived PH. Matsumiya and Kubo (2011) isolated a small bioactive peptide in a soybean-derived PH; this peptide, called 'root hair promoting peptide', increased the root hair numbers in *Brassica rapa* and adventitious root formation in tomato cuttings. Amino acids can also have a strong or weak auxin-like activity depending on the type of amino acid and its concentration in the PH (Ertani et al., 2009; Colla et al., 2014). Tryptophan, being the primary precursor in the biosynthesis of indole-3-acetic acid, has been associated with a high auxin-like activity of plant-derived PH (Colla et al., 2014). Moreover, Forde and Lea (2007) reported a significant effect of glutamate, even at very low concentrations, in changing root architecture by inhibiting primary root growth and increasing root branching near the root apex. The increase of root branching induced

by glutamate application can enhance the density of the root system improving the plant's ability to explore the soil volume and to uptake nutrients and water. Biostimulant action of PH can also result from biostimulant-mediated changes in microbial community of the rhizosphere as reported by Luziatelli et al. (2016) in a lettuce trial where the application of a plant derived-PH increased the population of bacteria species producing the auxin, 3-indoleacetic acid, and other auxin-like compounds.

Seaweed extract (SWE) are a complex mixture of bioactive compounds like polysaccharide, fatty acids, vitamins, phytohormones and mineral nutrients (Battacharyya et al., 2015). Several studies reported a stimulation of rhizogenesis and root growth after SWE application on cuttings or plants (Pacholczak et al., 2016; Vernieri et al., 2006). Interestingly, Vernieri et al. (2006) found that the addition to the nutrient solution of SWE significantly increased the root biomass of hydroponically-grown rocket especially under low nutrient availability. Recently, Hernández-Herrera et al. (2016) reported that polysaccharide-enriched extracts have a strong root growth-promoting activity suggesting that oligosaccharides can act as signaling molecules inducing changes in endogenous phytohormone metabolism of treated plants by a selective regulation of phytohormone metabolic genes. Moreover, polysaccharide-enriched extracts promoted the formation of longer roots in mung bean hypocotyl cuttings compared with the control and synthetic rooting hormone (Indole-3-butyric acid). Contradictory results have been also reported with no significant effects of SWE application on root growth (Rouphael et al., 2017a). These findings can be explained by the variation of chemical composition of SWE (arising from the type of seaweeds used, harvesting season, and extraction method), timing and rate of SWE application, crop type, phenological growth stage, and environmental conditions. Microbial-based biostimulants can also stimulate root growth (Rouphael et al., 2015b; Ruzzi and Aroca, 2015). Several studies reported that endophytic fungi such as AMF and *Trichoderma* spp. can promote plant rooting by stimulation of auxin production in mycorrhized roots or release of auxin-like compounds from *Trichoderma* hyphae (Colla et al., 2015b). Recently, Rubin et al. (2017), presenting the results of a meta-analysis to summarize 52 published articles on the effects of PGPR on growth and yield, reported that those bacteria induced an increase of root mass by 35% and 43% under well-watered and drought conditions, respectively. The increase in root growth induced by endophytic fungi and PGPR, as well as the development of an external mycorrhizal mycelium enhance the volume of soil accessible for plant nutrient uptake improving plants' capability to cope with low nutrient availability in soil.

PBs can also increase nutrient uptake by improving nutrient uptake activity of root system. Several studies reported that applications of HS, PHs, or SWE up-regulate the genes involved in the transport of nutrients. Jannin et al. (2012) reported that application of humic acids to *Brassica napus*, besides stimulating root growth, enhanced uptake of nitrogen and sulfate and this was associated with an upregulation in the roots of genes encoding for nitrate transporters (BnNRT1.1 and BnNRT2.1) and sulfate transporters (BnSultr1.1 and BnSultr1.2). Similar results were also obtained by the same authors (Jannin et al., 2013) after application of a brown seaweed-derived extract to *Brassica napus*.

On the contrary, application of PH containing high amount of free amino acids (especially glutamine) can inhibit root nitrate uptake because of the downregulation of

amino acids on root nitrate transporters (Colla et al., 2015a). Nazoa et al. (2003) reported the NRT2.1 gene which codes for a high-affinity nitrate transporter expressed essentially in *Arabidopsis* roots was upregulated by nitrates and down-regulated by amino acids. However, Liu and Lee (2012) reported that application of a mixture containing equal concentrations of seven amino acids (Ala, β -Ala, Asp, Asn, Glu, Gln, Gly) in radish and red pepper increased nitrate uptake under a high level of nitrate supply. These contradictory results can be explained by the different amino acid and nitrate concentrations used in the experiments. Plant biostimulants can also stimulate the activity of specific enzymes involved in root micronutrient uptake. Cerdán et al. (2013) reported that root application of a plant-derived PH product at low concentration enhanced root and leaf Fe(III)-chelate reductase activity promoting uptake and subsequent assimilation of Fe in tomato seedlings grown under a lime induced Fe-deficiency situation. In contrast, animal-derived PH caused severe plant-growth depression and non-positive effects on Fe nutrition of tomato seedlings. HS applications up-regulated the expression of genes encoding the Fe(III)-chelate reductase (Cannellas et al., 2015). Microbial-based biostimulants can also enhance plant nutrient uptake by promoting the activity of nutrient transporters and specific enzymes (Herman et al., 2012). Furthermore, Saia et al. (2015) reported that inoculation of unfertilized-durum wheat with AMF and AMF + PGPR upregulated the expression of nitrate transporter genes (NRT1.1, NRT2, and NAR2.2) and AMT1-type ammonium transporter genes in roots increasing the nitrogen in the aboveground biomass. On the contrary, the nitrate and ammonium transporter genes were downregulated when plants received 80 kg ha⁻¹ of N as hydrolysed leather meal-based fertilizer. Moreover, in unfertilized wheat roots, the expression of P transporters Pht1 and PT2-1 was upregulated by inoculation with AMF + PGPR, and inoculation with AMF up-regulated Pht2 compared to the non-inoculated control. Inoculation with PGPR and AMF+PGPR resulted in significantly higher aboveground P compared to the control and inoculation with AMF only when organic N was applied (Saia et al., 2015). In another study on potted pear plants grown under calcareous soil conditions, İpek et al. (2017) found that Fe nutrition of plants was improved after root inoculation with *Staphylococcus* and *Pantoea* strains due to their release of organic acids, which decreased the rhizospheric and apoplastic pH and thus increased root and leaf Fe(III)-chelate reductase activity.

Plant nutrient assimilation

PBs can also improve nutrient assimilation (such as nitrate, ammonium, phosphate, sulphate) stimulating the gene expression of enzymes functioning in the plant metabolism or simply as indirect effect of the increase plant nutrient uptake and transport. Using a microarray approach, Jannin et al. (2012) analyzed 31,561 genes and discovered that more than 300 genes were differently expressed after 3 days from application of HS whereas after 30 days from HS treatment the number of differently expressed genes was considerably reduced (102 genes in shoots and no differently expressed genes in roots). About 50% of the upregulated genes in shoots were involved in photosynthetic pathway and in nitrogen metabolism (e.g., nitrate reductase, nitrite reductase and the genes involved in amino acid metabolism). Moreover, 80% of the genes involved in sulfate metabolism (sulfate transporter, ATP sulfurylase and serine acetyltransferase) were also upregulated by HS. Similarly, Schiavon et al. (2008)

reported that the activity of enzymes involved in carbon metabolism (malate dehydrogenase, isocitrate dehydrogenase, citrate synthase) and nitrate assimilation (nitrate reductase, nitrite reductase, glutamine synthetase, glutamate synthase, aspartate aminotransferase) were stimulated by the protein hydrolysate (PH) application. Similarly, Ertani et al. (2009) found an increase of nitrate reductase activity, glutamine synthetase activity together with the high induction of glutamine synthetase isoforms in both leaves and roots of corn seedlings treated with two PHs, indicating a stimulation effect on nitrate assimilation. Nitrogen assimilation enzymes (e.g., nitrate reductase) were also stimulated by foliar applications of SWE in leaves of eggplant and creeping bentgrass (Zhang et al., 2010; Ramya et al., 2015). Interestingly, the stimulatory effect of SWE application was higher when the plants were grown at low nitrate concentration, suggesting that SWE may be beneficial for plant growth in adverse nutritional conditions such as in organic farming (Durand et al., 2003). Besides increasing nutrient uptake, arbuscular mycorrhizal fungi can enhance nutrient assimilation (formation of organic nitrogen compounds from inorganic nitrogen) as reported by Caravaca et al. (2007) in several experiments with two Mediterranean-autochthonous shrub species, where the inoculation with AMF enhanced nitrate reductase activity in roots and leaves. Similarly, Kohler et al. (2008) reported that inoculation of lettuce with AMF *Glomus intraradices* or the PGPR *Pseudomonas mendocina* induced increase of nitrate reductase activity in leaves under moderate drought conditions. Finally, Ruiz-Lozano and Azcon (1996) stated that the enhancement in nitrate reductase activity of mycorrhizal plants may be an indirect response to the improved nutrient status, particularly phosphorus. The increase in N-assimilating enzymes, such as nitrate reductase, may be due to the transport of considerable amount of NO₃⁻ to the host plant (Subramanian and Charest, 1999). However, in the case of inoculation with *P. mendocina*, the mechanism involved in increasing nitrate reductase activity has not been elucidated.

Conclusions

The overall objective of this review was to set a scientific frame to identify how the PBs treatments (substances and/or microorganisms) have the potential to enhance plant resilience to nutrient limitation typical of organic farming. Several studies in the scientific literature have documented the benefits of PBs application on growth, crop productivity, quality and tolerance to chemical soil stresses in particular nutrient deficiency of several vegetable crops but mostly under conventional farming. However, information about the potential benefits derived from applications of PBs in organic farming systems is completely missing. Therefore, more research is needed to elucidate the mode of action of PBs to overcome nutrient limitation throughout the improvement of nutrient availability and uptake, thus reducing the gap between organic and conventional yields. Furthermore, additional studies are also required for defining the optimal dose, application time and method for each species and environmental conditions. Finally, the regulatory situation of PBs in the European Union is still complex today, even if some implementing regulation (EU no. 354/2014; prohibition of the application of protein animal hydrolysates) related to the consumer safety concern has been recently introduced for organic crops.

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