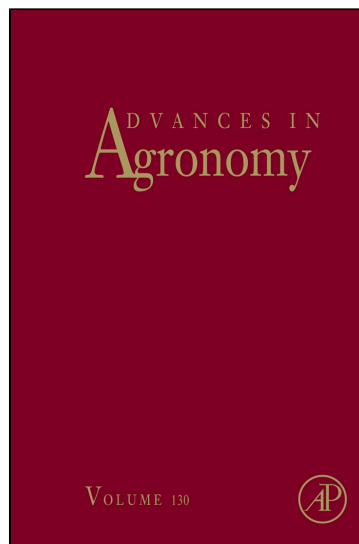


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The Use of Biostimulants for Enhancing Nutrient Uptake

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Abstract

Fertilizer use in modern agriculture is highly inefficient; much of the applied fertilizer is released into the environment, causing environmental degradation. One way in which fertilizer use can be reduced without damaging plant nutrition is to enhance crop uptake of nutrients through the use of biostimulants. A broad definition of plant biostimulants, including substances sometimes categorized as biofertilizers or biopesticides, is used throughout this review: "Plant biostimulants are substances or materials, with the exception of nutrients and pesticides, which, when applied to plants, seeds, or growing substrates in specific formulations, have the capacity to modify physiological processes in plants in a way that provides potential benefits to growth, development, or stress response." This definition includes a variety of substances, four of which will be reviewed in this article: seaweed extract, humic substances, amino acids, and plant-growth-promoting bacteria. We will concentrate on the positive effects of biostimulant application on plant nutrient uptake, and the underlying mechanisms, which include positive changes in soil structure or nutrient solubility, root morphology, plant physiology, and symbiotic relationships, will be discussed. Recommendations for future research directions include finding the most promising substances, isolating the active ingredients and clearly demonstrating the mechanisms by which they affect nutrient uptake. The beneficial effects and mechanisms must be consistently demonstrated in greenhouse and field experiments.

Abbreviations

AA	Amino acids or protein hydrolysates
AspAT	Aspartate aminotransferase
EDDHA	Ethylenediamine-N,N'-bis(2-hydroxyphenylacetic acid)
EDTA	Ethylenediaminetetraacetic acid
DTPA	Diethylene triamine pentaacetic acid

GDH	Glutamate dehydrogenase
GOGAT	Glutamate synthase
GS	Glutamate synthetase
HS	Humic substances
MDH	Malic dehydrogenase
NiR	Nitrite reductase
NR	Nitrate reductase
PGPB	Plant-growth-promoting bacteria
PM H⁺ATPase	Plasma membrane H ⁺ ATPase
SE	Seaweed extract



1. INTRODUCTION

The application of chemical fertilizers is an inexpensive and effective method of supplying crops with mineral nutrients (Chen, 2006). However, fertilizers are often washed from the field in the runoff (Daverede et al., 2004; Moe et al., 1967) or can become unavailable to the crops through chemical, physical, or biological transformation (Sánchez et al., 2001; Schachtman et al., 1998). To compensate for these processes, farmers need to apply more chemical fertilizer than the plant actually needs, and the remainder is often released into the environment, polluting the air and water (Vance, 2001). Furthermore, the industrial production of chemical fertilizers is an energy-intensive process that is known to significantly contribute to global CO₂ emissions (Vance, 2001).

Organic fertilizers, such as compost, sludge, or manure, have the advantage of making use of nutrients that are already available in the agro-ecosystem, and they require little input of energy to be processed. Furthermore, the mineral nutrients that are bound in organic materials may be more stable, and may therefore be washed away or released into the atmosphere less quickly (Estavillo et al., 1994; King and Torbert, 2007). However, organic fertilizers have the disadvantage of not supplying crops with nutrients in an easily absorbable, water-soluble form when the crops need them (Chen, 2006).

One method by which it is possible overcome this disadvantage is to grow crops with more robust root systems and higher nutrient-uptake efficiency, to ensure that they receive the nutrients when they need them despite their lower immediate availability when they are introduced in organic form. Alternatively, nutrients can be made more available by promoting certain types of organisms within the soil microbial community (Vessey, 2003). Both of these approaches can be achieved by introducing biostimulants to crop leaves, seeds, or soil as a means of stimulating root growth (Canellas et al., 2002; Khan et al., 2009; Zandonadi et al., 2007),

efficient root uptake (Pinton et al., 1999a), and beneficial microbial populations (Chen, 2006; Vessey, 2003).

The term biostimulant is relatively new and its use in the scientific community is still nebulous (du Jardin, 2012). One broad definition was introduced by du Jardin (2012): “Plant bio-stimulants are substances or materials, with the exception of nutrients and pesticides, which, when applied to plants, seeds or growing substrates in specific formulations, have the capacity to modify physiological processes in plants in a way that provides potential benefits to growth, development, or stress response.” Other workers differentiate between biostimulants, biofertilizers, and biopesticides by their direct hormonal effects (biostimulants) (Subler et al., 1998), indirect effects on nutrient availability (biofertilizers) (Orhan et al., 2006), and enhanced control of pathogens or pests (biopesticides) (Copping and Menn, 2000). Since many different mechanisms have been proposed for the plant’s positive reactions to the different substances discussed in this review, each can be called a biostimulant or biofertilizer. Therefore, we will employ the broader definition of biostimulants proposed by du Jardin (2012). Although this definition includes substances that help the plant overcome biotic or abiotic stresses, in this review, we will only be considering biostimulants that have positive effects on root growth, root efficiency, or nutrient uptake, traits that are useful in shifting from a chemical to organic fertilization regime.

Four major groups of biostimulants have been shown to affect root growth and nutrient uptake: (1) humic substances (HS), (2) protein hydrolysate and amino acid formulations (AA), (3) seaweed extract (SE), and (4) plant-growth-promoting microorganisms. Although mycorrhizal fungi are included in group 4, they are not treated in this review because of the many articles and reviews that have already been written on the topic (see for example: Bolan, 1991; Cavagnaro, 2008; Marschner and Dell, 1994). Thus only plant-growth-promoting bacteria (PGPB) will be discussed from this group.



2. HUMIC SUBSTANCES

2.1 Introduction to HS

HS are heterogeneous organic molecules that form in the soil as byproducts of microbial metabolism of dead organic matter (Nardi et al., 2007). HS are one of the most common organic substances on Earth (Sutton and Sposito, 2005), and make up 60% of the organic matter in the world’s soils (Muscolo et al., 2007). In the past, HS were thought to be large linked polymers of organic molecules. However, the emerging

consensus is that HS are made up of many small organic molecules that are held together by hydrophobic interactions and hydrogen bonds (Piccolo, 2002; Simpson et al., 2002; Sutton and Sposito, 2005).

HS can be extracted from many different sources, including soils (Nardi et al., 2000; Varanini et al., 1993; Zandonadi et al., 2007), municipal waste (Ayuso et al., 1996), vermicomposts and earthworm casts (Canellas et al., 2002; Russell et al., 2006), various coal deposits (Kulikova and Perminova, 2002), peat (Ayuso et al., 1996; Schmidt et al., 2007), and Leonardite (Nikbakht et al., 2008).

HS can be applied to the plant in a number of ways, including foliar applications (Katkat et al., 2009; Yildirim, 2007), in the irrigation water (Salman et al., 2005), and direct application to the soil (Katkat et al., 2009).

2.2 Characterization of HS

HS are often divided into fractions according to their molecular weight (Nardi et al., 2007; Quaggiotti et al., 2004; Russell et al., 2006; Varanini et al., 1993). The lower molecular weight fractions tend to have greater positive biological effects on plants (Piccolo et al., 1992; Varanini et al., 1993), but this is not always the case (Muscolo et al., 2007; Nardi et al., 2007). Furthermore, the true molecular weight is not easy to determine, since HS are thought to be made up of many different-sized molecules that interact with one another on a supramolecular scale (Piccolo, 2002; Simpson et al., 2002; Sutton and Sposito, 2005).

A number of different methods have been used to characterize HS, including three spectroscopic ones: DRIFT (diffuse reflectance infrared Fourier transform), $^1\text{H-NMR}$ (H^1 nuclear magnetic resonance) (Muscolo et al., 2007), and $^{13}\text{C-NMR}$ (Canellas et al., 2010). In recent studies, the specific chemical structure of the HS rather than the sizes of their molecules have been shown to affect the biological activity of the HS (Canellas et al., 2010; Muscolo et al., 2007).

2.3 Effects of HS on Plant Growth

HS have a number of positive effects on plant growth, including increased biomass (Ayuso et al., 1996; Lee and Bartlett, 1976), increased number of fruits and flowers (Arancon et al., 2006), and improved fruit quality (Yildirim, 2007).

2.3.1 Effects of HS on Nutrient Uptake

HS have a positive effect on nutrient uptake (see Table 1). There have been a number of studies showing that HS increase NO_3 uptake (Albuzio et al.,

Table 1 Examples of positive effects of humic substance (HS) application on plant nutrition

Plant	Nutrients positively affected by HS	Parent material of HS	References
Barley	NO ₃	Soil	Albuzio et al. (1986)
Barley	NO ₃	Coal	Piccolo et al. (1992)
Barley	NO ₃	Soil	Nardi et al. (2000)
Maize	NO ₃	Earthworm feces	Quaggiotti et al. (2004)
Barley	N, P, Mn, Cu, Zn, Fe	Sewage sludge, compost, leonardite, and peat	Ayuso et al. (1996)
Maize	P, Fe	Cow manure, compost, peat, and soil	Lee and Bartlett (1976)
Maize	N, Zn	Soil	Tan and Nopamornbodi (1979)
Melon, soybean, and rye grass	Zn, Fe	Peat and leonardite	Chen et al. (2004)
Maize	Cu, Zn, Mn	Leonardite	Çelik et al. (2011)
Tomato	Fe	Lignite (brown coal)	Sánchez-Sánchez et al. (2005)
Grape	Fe	Not specified	Sánchez-Sánchez et al. (2006)

1986; Nardi et al., 2000; Piccolo et al., 1992; Quaggiotti et al., 2004). These studies were relatively short term, testing NO_3 uptake, gene transcription, and activities of the proteins involved in NO_3 uptake and assimilation over the course of 16–48 h in very young plants. Tan and Nopamornbodi (1979) measured the effects of HS derived from soil on the nutrient uptake of maize plants over the course of a growing season. Whereas N and Zn uptake were improved at certain doses, P uptake was negatively affected irrespective of the HS dose, while the uptake rates of other minerals were not significantly affected. Ayuso et al. (1996) showed that HS from a number of different parent materials can improve the uptake of total N as well as other nutrients, such as P, Mn, Cu, Zn, and Fe in barley over the course of an entire growing season. Plant acquisition of each of these nutrients was affected differently by different HS doses; some doses affected certain minerals positively and others negatively. Lee and Bartlett (1976) found that HS greatly improve P and Fe uptake in maize when they are applied to soils with little organic material. When applied to soils with high concentrations of organic material, the positive effects were small or nonexistent, probably because the background levels of HS were already high in those soils. Some studies found positive effects of HS on micronutrient uptake, specifically in alkaline soils or alkaline nutrient solutions where micronutrients are often limiting (Çelik et al., 2011; Chen et al., 2004; Sánchez-Sánchez et al., 2005, 2006).

2.4 Mechanisms by Which HS Affect Nutrient Uptake

HS improve plant nutrition by affecting soil processes and by directly affecting the plant's physiology. The mechanisms that affect the soil processes include: (1) improvement of the soil structure, (2) improvement of micronutrient solubility in the soil. Direct effects on the plant's physiology include: (3) changes in root morphology, (4) an increase in root activity of H^+ ATPase, and (5) an increase in the activity of NO_3 -assimilation enzymes.

2.4.1 HS Improve Soil Structure

HS improve plant nutrition by improving the soil structure. Piccolo et al. (1997) found that amending the soil with HS increases aggregate stability. They attributed this phenomenon to the HS' ability to form clay–humic complexes with hydrophilic components oriented toward the center of the aggregate and hydrophobic components facing outward. This reduces water infiltration into the aggregates, making them more stable in wetting and drying cycles. Improved aggregate stability leads to improved soil

aeration, facilitated root penetration, greater water availability to the plant, and less soil erosion, which indirectly contribute to enhanced nutrient uptake (Amezketta, 1999; Bronick and Lal, 2005). However, improved aggregate stability does not explain the observed improvement in plant nutrition in hydroponic systems (Chen et al., 2004), or when the HS are applied to the foliage rather than the soil (Katkat et al., 2009).

2.4.2 HS Improve Solubility of Micronutrients and P

Under some circumstances, micronutrients and P are highly insoluble. HS added to the nutrient solution enhance Fe and Zn solubility by forming metal–humic complexes (Chen et al., 2004). The Fe–humic complexes are available to plants, regardless of whether they use strategy I (dicots and nongraminaceous monocots) or strategy II (graminaceous monocots) for Fe mobilization (Cesco et al., 2000; Chen et al., 2004; Pinton et al., 1999b). In fact, the increased growth observed in plants treated with HS may be attributed to increased Fe availability (Chen et al., 2004; Pinton et al., 1999b). Application of the water-soluble fraction of HS increased the solubility of Fe-hydroxides, as well as their mobility in the soil (Cesco et al., 2000). HS have been shown to be effective replacements for artificial chelates of Fe such as ethylenediamine-*N,N'*-bis(2-hydroxyphenylacetic acid) (EDDHA) in tomato, lemon trees, and grapevines grown in calcareous soils (Sánchez-Sánchez et al., 2002, 2005, 2006). HS also increase the activity of plasma membrane (PM) H⁺ATPase (Pinton et al., 1999a), which could lead to rhizosphere acidification and hence to increased solubility of micronutrients. HS increase the availability of P by interfering with the formation of nonsoluble Ca-phosphates (Delgado et al., 2002). This explains the increased efficiency of P use when soluble phosphate fertilizers are applied to soils that have been amended with organic materials (Delgado et al., 2002).

2.4.3 HS Change Root Morphology

Malik and Azam (1985) showed greater root development of wheat seedlings grown in distilled water supplemented with HS versus distilled water alone. Canellas et al. (2002) and Zandonadi et al. (2007) showed that HS derived from earthworm compost increase lateral-root proliferation and elongation in maize. They both attributed this effect to the auxin-like activity of HS, which stimulates PM H⁺ATPase, thereby stimulating cellular growth. Schmidt et al. (2007) showed that water-soluble HS derived from peat cause an increase in root-hair density in *Arabidopsis*, but they ruled

out the involvement of auxin-like activity by showing that HS cannot save an auxin-deficient mutant. An increase in lateral-root and root-hair development increases the surface area of the root, which would explain the increased nutrient uptake induced by HS.

2.4.4 HS Stimulate H^+ ATPase and NO_3 -Assimilation Enzymes

As already mentioned, HS are known to stimulate PM H^+ ATPase (Canellas et al., 2008; Nardi et al., 2000; Pinton et al., 1999a; Quaggiotti et al., 2004). Zandonadi et al. (2010) showed that this stimulation involves auxin-like activity of the HS and nitric oxide (NO) signaling, by demonstrating that auxin inhibitors and NO-scavenging molecules inhibit PM H^+ ATPase stimulation by HS. By stimulating the PM H^+ ATPase, HS acidify the rhizosphere, causing the $NO_3^- H^+$ symport system to work more effectively. The cumulative result is that the plant absorbs more NO_3^- (Pinton et al., 1999a; Quaggiotti et al., 2004; Zandonadi et al., 2010).

HS also increase the rate of NO_3^- assimilation by causing the plant to upregulate the enzymes involved in this process. Albuzio et al. (1986) found that barley plants incubated with HS derived from soil and fractionated in various ways had increased nitrate reductase (NR), glutamate dehydrogenase (GDH), and glutamate synthetase (GS) activity. While some fractions had more of a stimulatory effect than others, the effect did not correlate well with fraction size. Muscolo et al. (1999) found an increase in GDH, GS, and malic dehydrogenase (MDH) activity in carrot cells treated with HS derived from worm castings. They attributed this stimulatory effect to the auxin-like substances found in the castings. Vaccaro et al. (2009) found that the hydrophilic and least structurally complex HS derived from compost have a stimulatory effect on many NO_3^- -assimilation enzymes, including NR, nitrite reductase (NiR), GS, glutamate synthase (GOGAT), and aspartate aminotransferase (AspAT).



3. AMINO ACIDS

3.1 Introduction to AA

AA are a large family of biological compounds that contain an amine functional group and a carboxylic acid functional group. There are only 20 AA involved in protein building, but there are 250 more that are known to have diverse functions in plants, including protection from biotic and abiotic stresses, signaling, N storage, and chelation of metals as phytochelatins (Vranova et al., 2011). Commercially available AA biostimulants are mostly

mixtures of different AA and short peptides, rather than pure substances (du Jardin, 2012). These mixtures, called protein hydrolysates, are derived from the hydrolysis of proteins from plant (Schiavon et al., 2008), animal (Maini, 2006) and microbial (du Jardin, 2012) sources, often from industrial and agricultural waste products such as crop residues (du Jardin, 2012), animal skin (Vasileva-Tonkova et al., 2007), feathers (Grazziotin et al., 2007; Jie et al., 2008), and blood (Polo et al., 2006). Protein hydrolysates are marketed as plant biostimulants that can be applied as a foliar spray, soil drench, or seed treatment (du Jardin, 2012; Maini, 2006). The scientific literature discusses both pure AA (Ghasemi et al., 2012; Rodríguez-Lucena et al., 2010; Yuan et al., 2013; Zhou et al., 2007) and protein hydrolysates (Ertani et al., 2009; García-Martínez et al., 2010; Maini, 2006; Schiavon et al., 2008). In this review we will refer to both protein hydrolysates and pure AA as “AA,” and specify the name of the pure AA when it is relevant.

3.2 Absorption of AA by Plants

Plants can absorb AA directly into the roots when they are dissolved in the mass flow of water into the xylem (Biernath et al., 2008), through specific transporters in the roots (Näsholm et al., 2009) or via diffusion into the leaves (Kolomaznik et al., 2012; Pecha et al., 2011). Plants can utilize AA as a source of N, and under some circumstances, in certain plants, AA are the main source of N (Schimel and Chapin, 1996). Strictly speaking, when AA are used as a source of N, they do not fit du Jardin's, 2012 definition of biostimulant, which specifies that nutrients are not considered biostimulants. However, the dose at which AA are usually applied as biostimulants is so low that their positive effects cannot be attributed to the increase in N availability. For example, Schiavon et al. (2008) applied 0.1 and 0.01 ppm AA which contained 2.29% N (a total of 0.016–0.16 $\mu\text{mol L}^{-1}\text{N}$) to a nutrient solution with 600 $\mu\text{mol L}^{-1}\text{N}$, and Zhou et al. (2007) applied between 10 and 100 $\mu\text{mol L}^{-1}$ pure AA to a nutrient solution with 5000 $\mu\text{mol L}^{-1}\text{N}$. Furthermore, mechanisms other than improved N nutrition have been implicated in the beneficial effects of AA application (Ghasemi et al., 2012; Maini, 2006; Schiavon et al., 2008; Zhou et al., 2007).

3.3 Effects of AA on Plants

AA application has been shown to increase biomass production (Shehata et al., 2011), help protect plants against biotic (Cohen and Gisi, 1994) and abiotic (Maini, 2006; Polo et al., 2006) stresses, and increase the antioxidant content of the leaves (Ardebili et al., 2012).

3.3.1 Effects of AA on Nutrient Uptake

Application of exogenous AA to plant leaves and roots has been shown to increase nutrient uptake and nutrient-use efficiency for both macro- and micronutrients (see Table 2). A commercial mixture of AA was shown to increase corn yield, even when the N-fertilization rate was cut in half (Maini, 2006). Ca can also be better utilized by the plant when it is applied together with AA, and mixtures of AA and Ca are used to reduce Ca deficiency in apples and tomatoes (Maini, 2006). However, Otero et al. (2006) found that a mixture of AA and Ca was not effective in kiwifruit and recommended finding other methods of Ca fertilization. AA were also shown to increase the efficiency of foliar-applied micronutrients, and mixtures of FeSO₄ and AA applied as a foliar spray were shown to be effective against chlorosis in grapevine (Maini, 2006).

3.4 Mechanisms by Which AA Affect Nutrient Uptake

AA application can improve plant nutrition by affecting soil processes and by affecting the plant's physiology directly. The mechanisms affecting the soil processes include: (1) promotion of beneficial microbial communities and nutrient mineralization in the soil, (2) improvement of micronutrient solubility in the soil through chelation and reduction of micronutrients. The mechanisms that affect the plant's physiology directly include: (3) improvement of micronutrient mobility in the plant, (4) changes in root morphology, (5) increased activity of NO₃-assimilation enzymes.

3.4.1 AA Increase Soil Microbial Activity

AA application to the soil increases soil microbial activity, which can improve the soil's physical and chemical attributes (Garcia-Martinez et al., 2010). Specifically, the increased bioactivity in the soil causes a quicker breakdown of organic matter, which transforms organic nutrients into plant-available mineral forms (Garcia-Martinez et al., 2010).

3.4.2 AA Chelate Micronutrients

AA can chelate metals such as Fe, Zn, Mn, Cu, making them more readily absorbable through the roots and leaves via specific transporters, such as lysine histidine transporter 1 (LHT1), amino acid permease 1 (AAP1) and AAP5 (Ghasemi et al., 2012; Jie et al., 2008). In nature, plants often secrete specific nonprotein AAs known as phytosiderophores from their roots into the soil to improve micronutrient availability (Dakora and Phillips, 2002; Kinnersley, 1993). For this reason, many micronutrient foliar sprays (for

Table 2 Examples of positive effects of amino acid (AA) application on plant nutrition

Plant	Nutrients positively affected by AA	Parent material or specific AA	References
Maize	N	Animal epithelial tissue	Maini (2006)
Maize	NO ₃	Alfalfa protein hydrolysate and meat flour (hydrolysate)	Ertani et al. (2009)
Maize	NO ₃	Alfalfa protein hydrolysate	Schiavon et al. (2008)
Tomato	Fe, Zn, N	Histidine, glycine, and arginine	Ghasemi et al. (2012)
Rice	Fe, Zn, Cu, Mn	Chicken feather hydrolysate	Jie et al. (2008)
Pear	Fe, Zn	Commercial mixture of amino acids	Koksal et al. (1999)
Soybean	Fe	3 commercial mixtures with the main components glycine, glycine + glutamate, and glycine + arginine. These mixtures also contained 3–15% polypeptides	Rodríguez-Lucena et al. (2010)
Maize	Cu	Cysteine	Zhou et al. (2007)
Rice	Fe, Zn	Nicotianamine and other unspecified amino acids	Yuan et al. (2013)

example, Koksal et al., 1999; Rodríguez-Lucena et al., 2010) and hydroponic solutions (for example, Ghasemi et al., 2012) contain AA mixtures.

3.4.3 AA Reduce Micronutrients

Specific AAs may also increase the availability of micronutrients by acting as a reductant. Zhou et al. (2007) showed that exogenous application of cysteine to maize roots in a hydroponic solution causes an increase in Cu uptake. They hypothesized that the cysteine acts as a reductant, changing Cu II to Cu I, which may be more available to the roots.

3.4.4 AA Improve Internal Translocation of Micronutrients

AA chelates are also important for the translocation of micronutrients within the plant. There have been a large number of studies showing that nicotianamine, a nonprotein AA, is responsible for the translocation of micronutrients in the phloem (Curie et al., 2009; Schmidke and Stephan, 1995; Stephan et al., 1994). Nicotianamine has also been shown to have positive effects on plant physiological processes, even when applied exogenously, and it can therefore be considered a biostimulant. Specifically, exogenous application of nicotianamine increased the translocation of Zn and Fe to the grains of rice plants, which has important implications for human nutrition (Yuan et al., 2013).

3.4.5 AA Affect Root Morphology

Exogenous application of AA has also been shown to have an effect on root morphology. Specifically, L-glutamate application to the root inhibited primary-root growth and stimulated root branching (Walch-Lui et al., 2006). It also stimulated root-hair development close to the root tip (Walch-Lui et al., 2006). This effect was specific to L-glutamate, and did not occur in response to applications of 21 other AA, including D-glutamate (Walch-Lui et al., 2006). Tryptophan was shown to have a different stimulatory effect on root growth, which the authors attributed to the fact that this AA is a precursor for auxin production (Walch-Lui et al., 2006).

3.4.6 AA Stimulate NO₃-Assimilation Enzymes

AA have also been shown to stimulate the NO₃-assimilation enzymes through hormonal action. Maini (2006) reviewed a number of studies that showed that a commercial AA foliar spray increases NR activity. Schiavon et al. (2008) conducted an in-depth study showing that AA derived from a hydrolysate of alfalfa proteins applied at a rate of 0.1 mg L⁻¹ have a significant impact on the enzymes associated with NO₃ assimilation in *Zea mays*

plants grown in full Hoagland solution. The enzymes measured in the root and leaves included NR, NiR, GS, and GOGAT. The AA treatment led to a statistically significant increase in all of the NO_3 -assimilation enzymes measured, in both the root and the shoot. The overall N concentration was unchanged by the treatment, but the NO_3 concentration decreased, indicating that the treatment caused the plants to assimilate the NO_3 more rapidly. Enzymes involved in C metabolism were also positively affected, indicating that C and N metabolism are regulated together, helping the plant regulate the C:N balance. Ertani et al. (2009) studied the effects of AA mixtures from protein hydrolysates of alfalfa or meat meal on NR and GS; the AA mixtures had stimulatory effects, regardless of the origin of the AA. They also measured NO_3 in the roots and shoots, and found that its concentration is significantly decreased by the application of protein hydrolysate compared to controls. This indicated that NO_3 assimilation is stimulated by the treatments (Ertani et al., 2009). Both Schiavon et al. (2008) and Ertani et al. (2009) attributed the observed effects on the NO_3 -assimilation enzymes to the auxin-like and gibberellin-like activities of the protein hydrolysates. In both studies, auxin-like and gibberellin-like activities were confirmed using bioassays for the plant-based (Schiavon et al., 2008) and animal-based (Ertani et al., 2009) protein hydrolysates.



4. SEAWEED EXTRACT

4.1 Introduction to SE

Seaweed has been applied as a fertilizer in coastal regions for centuries (Craigie, 2011). The first method for liquefying seaweed for agricultural use was patented in 1912 (Booth, 1969). Liquefied SE is usually manufactured from *Ascophyllum nodosum*, a brown seaweed that is commonly found in the North Atlantic, although other species, such as *Durvillaea antarctica*, *Durvillaea potatorum*, *Macrocystis pyrifera*, and *Ecklonia maxima* are also used (Khan et al., 2009). The methods for liquefying seaweed are often proprietary, but they mostly involve physical disruption of the seaweed and/or its chemical digestion using alkalis or acids (Craigie, 2011). SE also contains some plant nutrients, and when applied to plants grown in a nutrient-deficient medium, the simple availability of these nutrients may improve growth and nutrient uptake. For example, Becket and van Staden (1989) observed a positive effect on K uptake, even when the SE was “ashed” (heated at 450 °C for 1 h). The authors surmised that this effect was simply caused by the K in the SE, which was present at a rate of 18 mg L⁻¹ of a root drench applied three times

during the growing season. Strictly speaking, when SE is used as a source of K, it does not fit [du Jardin's \(2012\)](#) definition of biostimulants, which specifically excludes nutrients.

4.2 Characterization of SE

SE is a heterogeneous substance that can be characterized by its parent material ([Khan et al., 2009](#)), the pH of the extraction solution ([Booth, 1969](#)), or $^1\text{H-NMR}$ spectroscopy ([Craigie et al., 2008](#)). SE have been shown to contain plant hormones such as auxins, cytokinins, and abscisic acid ([Khan et al., 2009](#)). SE also contain AA ([Khan et al., 2009](#)), whose effects on plant nutrition were discussed in Section 3.

4.3 Effects of SE on Plants

SE have been shown to increase plant growth ([van Staden et al., 1994](#)), chlorophyll levels ([Blunden et al., 1997](#)), flowering and yield ([Crouch et al., 1990](#); [Eris et al., 1995](#)), and seed germination ([Demir et al., 2006](#); [Kumar and Sahoo, 2011](#)). They increase the success of in vitro propagation ([Vinoth et al., 2012](#)) and enhance plant protection against pathogens and pests ([Hankins and Hockey, 1990](#); [Jayaraman et al., 2011](#); [Khan et al., 2009](#); [Klarzynski et al., 2003](#); [Loureiro et al., 2012](#); [Mercier et al., 2001](#)).

4.3.1 Effects of SE on Nutrient Uptake

SE has been shown to have a positive impact on plant nutrient uptake (see [Table 3](#)). [Turan and Köse \(2004\)](#) tested the effects of foliar application of three different commercial SE on nutrient uptake in a 1-year-old grapevine planted in perlite medium. When the vines were grown with a supply of mineral nutrients at optimal or high levels, all three SE induced significant improvement in macronutrient and micronutrient concentrations in the leaves, but no effect was seen when plants were grown at suboptimal mineral nutrition. Interestingly, plant acquisition of Cu was shown to be more strongly affected by the application of SE than by the concentration of Cu in the growth medium.

[Rathore et al. \(2009\)](#) found that foliar application of SE obtained from the red alga *Kappaphycus alvarezii* increases the grain concentration of N, P, K, and S by up to 36%, 61%, 49% and 93%, respectively in soybeans grown under rainfed conditions. The nutrient concentration in the grain showed a clear correlation with the SE dosage, with the highest concentration found in plants receiving the highest dosage. The nutrient levels in the straw were affected either positively or negatively, depending on the SE dosage.

Table 3 Examples of positive effects of seaweed extract (SE) application on plant nutrition

Plant	Nutrients positively affected by SE	Seaweed type	References
Grapevine	N, P, K, Ca, Mg, Fe, Zn, Mn, Cu	<i>Ascophyllum nodosum</i> (Maxicrop) and other commercial extracts of unknown source (Proton and Algipower)	Turan and Köse (2004)
Soybean	N, P, K, S	<i>Kappaphycus alvarezii</i>	Rathore et al. (2009)
Wheat	K	<i>Ecklonia maxima</i> (Kelpak)	Beckett and van Staden (1989)
Lettuce	Ca, K, Mg	<i>Ecklonia maxima</i> (Kelpak)	Crouch et al. (1990)
Cucumber	P	<i>Ecklonia maxima</i> (Kelpak)	Nelson and van Staden (1984)
Strawberry	Fe	<i>Ascophyllum nodosum</i> (Actiwave)	Spinelli et al. (2010)

Crouch et al. (1990) measured the effects of a commercial SE on growth, as well as Ca, Mg, and K uptake. They found that the growth of nutrient-stressed lettuce was not improved by root flushing with SE, but that nutrient uptake and plant growth were positively affected when the SE was applied to lettuce plants that were also receiving a highly concentrated nutrient solution. Nelson and van Staden (1984) applied the same commercial SE to cucumber plants grown in pine bark medium. The SE was applied at a low dosage as a root drench and as a foliar treatment. The seaweed treatments caused the roots to grow significantly larger. They measured the N and P concentration in the leaves, and found that the weekly spray of SE increased the foliar P concentration by 20%, and that the root drench caused a statistically insignificant increase of 5% in foliar P. The root drench and the foliar spray caused statistically significant decreases in leaf N concentration (−15% and −18%, respectively). The authors attributed the drop in foliar N to a large N sink in the roots caused by the SE treatments, but they did not measure the root N to confirm this hypothesis.

4.4 Mechanisms by Which SE Affects Nutrient Uptake

SE improves plant nutrition by affecting soil processes and by affecting the plant's physiology directly. The mechanisms that affect soil processes include: (1) improvement of soil structure, (2) improvement of micronutrient solubility in the soil. The mechanisms that affect the plant's physiology directly include: (3) changes in root morphology, and (4) increased root colonization by arbuscular mycorrhizal fungi.

4.4.1 SE Improves Soil Structure

SE supports plant nutrition by improving the soil structure. Brown seaweed contains large amounts of polysaccharides such as alginates and fucoidans, which bond with the metallic ions in the soil to produce a gel that helps hold water and maintain an aggregate structure (Khan et al., 2009). This helps the plant grow a robust root system, which in turn can increase nutrient uptake. However, improved soil structure does not explain the observed improvement in plant nutrition in hydroponic systems (e.g., Vernieri et al., 2006), nor the observed improvement when SE is applied to the foliage rather than the soil (e.g., Rathore et al., 2009).

4.4.2 SE Improves Micronutrient Solubility

There have not been many studies on the chelating effects of SE on micronutrients, although it is possible that the large organic molecules in the SE

can chelate micronutrients and render them more available. One recent study involving SE and the solubility of micronutrients showed that a commercial extract of *A. nodosum* can be used to replace sequestrene, a standard iron chelate (Spinelli et al., 2010).

4.4.3 SE Affects Root-to-Shoot Ratio

Application of SE to either leaves or roots has been shown to lead to an increase in root mass or root-to-shoot ratio in various different crops, such as cucumber (Nelson and van Staden, 1984), barley (Steveni et al., 1992), rocket (Vernieri et al., 2006), tomatoes (Crouch and van Staden, 1992; Featonby-Smith and van Staden, 1983) and strawberries (Spinelli et al., 2010), and in a mung bean bioassay (Stirk and van Staden, 1997). This effect is believed to be a result of auxins (Crouch and van Staden, 1992; Khan et al., 2009) or cytokinins (Steveni et al., 1992) in the SE. A larger, more robust root system contributes to nutrient uptake (Khan et al., 2009).

4.4.4 SE Promote Symbiotic Relationship Between Mycorrhizal Fungi and Roots

Arbuscular mycorrhizal fungi are associated with the roots of most terrestrial plants, and they play an important role in nutrient uptake. SE has been shown to encourage arbuscular mycorrhizal fungal growth and infection rates (Khan et al., 2009). Kuwada et al. (2006) found that extracts from the green and red algae *Gracilaria verrucosa*, *Gelidium amansii*, *Eucheuma cottonii*, and *Chlorella pyrenoidosa* significantly stimulate the growth of the arbuscular mycorrhizal fungi *Gigaspora margarita* and *Glomus caledonium* both in vitro (on petri dishes) and in vivo (on passionfruit cuttings and papaya seedlings).



5. PLANT-GROWTH-PROMOTING BACTERIA

5.1 Introduction to PGPB

PGPB are found in the bulk soil or rhizosphere and promote plant growth under some conditions (Bashan and de Bashan, 2005). PGPB belong to diverse genera and promote plant growth in various different ways (see Table 4 for examples). PGPB have been shown to have a number of positive effects on plant growth, including pathogen control (Bashan and de Bashan, 2005), increased salt tolerance (Alavi et al., 2013), increased resistance to heavy metals and other toxins (Lucy et al., 2004), increased growth and yield (Alam et al., 2011; Lucy et al., 2004), and enhanced plant nutrition

Table 4 Examples of positive effects of plant-growth-promoting bacteria (PGPB) application on plant nutrition

Crop	PGPB	Nutrients positively affected by PGPB	Main mechanisms	References
Fingermillet, maize, amaranth, buckwheat, frenchbean	<i>Bacillus</i> sp.	P	P solubilization	Pal (1998)
Maize	<i>Rhizobium leguminosarum</i> bv. <i>phaseoli</i>	P	P solubilization	Chabot (1998)
<i>Ficus benjamina</i>	<i>Bacillus coagulans</i> , <i>Trichoderma harzianum</i>	P	Positively affect mycorrhizal fungi	Srinath et al. (2003)
Wheat	<i>Azotobacter chroococcum</i>	P	P solubilization and hormone production	Kumar and Narula (1999)
Wheat	<i>Bacillus circulans</i> and <i>Cladosporium herbarum</i>	P	P solubilization and interaction with arbuscular mycorrhizal fungi	Singh and Kapoor (1999)
Tomato	<i>Enterobacter agglomerans</i>	N, P	P solubilization and positive effect on mycorrhizal fungi	Kim et al. (1997)
Maize and sunflower	Unknown	Fe	Fe solubilization	Masalha et al. (2000)
Barley	<i>Pseudomonas putida</i>	Fe	Fe chelation	Duijff et al. (1994)
Mung bean	<i>Pseudomonas</i> sp.	Fe	Fe chelation	Sharma et al. (2003)
Maize	<i>Pseudomonas alcaligenes</i> , <i>Bacillus polymyxa</i> , <i>Mycobacterium phlei</i>	N, P,K	Hormonal effects on root growth	Egamberdiyeva (2007)
Peas	<i>Rhizobia</i>	N	N fixation	Johnston and Beringer (1976)
Kenya clover	<i>Rhizobia</i>	N	N fixation	Moore and Britten (1964)
Chickpea	<i>Rhizobia</i>	N	N fixation	Yadav et al. (2011)

(Richardson et al., 2009; Vessey, 2003). In this review, we focus on the effects of PGPB on plant nutrition.

PGPB can be inoculated onto the seed or directly into the soil (Smith, 1992), and are usually mixed with a carrier material such as peat, manure, compost, sawdust, or vermiculite (Smith, 1992). These carriers provide a favorable environment for the PGPB when they are initially introduced to the often hostile soil environment (van Veen et al., 1997). Proper storage conditions and a good understanding of the local soil ecology are essential for successful inoculation (van Veen et al., 1997).

5.2 Mechanisms by Which PGPB Affect Plant Nutrition

There have been many studies showing the positive effects of PGPB on plant nutrition (see Table 4). Depending on the underlying mechanisms, they can affect the uptake of a single nutrient or a broad spectrum of nutrients. For example, PGPB that affect mycorrhizal symbiosis will affect the uptake of many nutrients, whereas PGPB affecting uptake through N fixation, or P or Fe solubilization, will only affect the uptake of those specific nutrients.

5.2.1 Some PGPB Fix N

One of the earliest PGPB mechanisms discovered was N fixation, and commercial inoculations of N-fixing Rhizobia, which form symbiotic relationships with legumes, have been available since the 1890s (Vessey, 2003). Mixed inoculations of endophytic diazotrophic bacteria such as *Gluconacetobacter diazotrophicus*, *Burkholderia tropica*, *Azospirillum amazonense*, *Herbaspirillum rubrisubalbicans*, and *Herbaspirillum seropedicae* have also been shown very effective at promoting N fixation in sugar cane (Oliveira et al., 2009). However, except for sugar cane, the use of nonrhizobial N-fixing PGPB in other nonlegumes has met with limited success (Vessey, 2003). In fact, many free-living N-fixing PGPB which were thought to improve plant growth because of their ability to fix N have since been shown to promote plant growth through other mechanisms. Many reviews have been written on the topic of N-fixing PGPB, for example, Hardarson (1993), Provorov and Tikhonovich (2003), Schubert (1995), and Vance (2001).

5.2.2 Some PGPB Solubilize P

Some PGPB have been shown to improve plant nutrition through P solubilization (Vessey, 2003). The total concentration of P in agricultural soils usually ranges between 400 and 1200 mg kg⁻¹. However, only 1 mg kg⁻¹ is generally present in available forms such as HPO₄⁻ and

$\text{H}_2\text{PO}_4^{-2}$ (Rodríguez and Fraga, 1999). The nonsoluble P in agricultural soils is present in inorganic and organic forms. The nonsoluble inorganic forms account for about 20–50% of the total soil P (Richardson, 2001), usually in the form of PO_4^- ions. These ions are either adsorbed onto the positively charged constituents of the soil, or they form poorly soluble precipitates with Fe, Al, or Ca, depending on the pH (Richardson, 2001). The nonsoluble organic P accounts for 50–80% of the total soil P (Richardson, 2001), and is comprised of phosphate esters, inositol phosphates, and large, uncharacterized organic molecules (Richardson, 2001).

Bacteria use a number of strategies to solubilize the nonsoluble inorganic and organic P compounds. To solubilize inorganic P, bacteria often synthesize organic acids such as gluconic and citric acids, which chelate the insoluble compounds and lower the pH, both of which increase P solubility (Gamalero and Glick, 2011). Another mechanism is to simply release protons, which lowers the pH and increases solubility without the help of chelates (Gamalero and Glick, 2011). Bacteria also increase P availability by mineralizing organic P (Gamalero and Glick, 2011).

The ability to solubilize P is common in rhizospheric bacteria (Richardson, 2001), and many such bacteria have been isolated, including those from the genera *Pseudomonas*, *Bacillus*, *Rhizobium*, *Burkholderia*, *Enterobacter*, *Streptomyces*, *Achromobacter*, *Agrobacterium*, *Micrococcus*, *Aerobacter*, *Flavobacterium*, and *Erwinia* (Gamalero and Glick, 2011; Rodríguez and Fraga, 1999). However, not all bacteria with the ability to solubilize P benefit the plant by increasing P uptake when inoculated into the soil (Richardson, 2001). It may be that the mechanisms that work well in laboratory culture do not work as well under soil conditions, or that the organisms themselves do not thrive in the soil (Richardson, 2001).

5.2.3 Some PGPB Solubilize Fe

Like P, Fe is also abundant in soils but mostly in the nonsoluble Fe III oxide form, such as hematite, goethite, and ferrihydrite (Masalha et al., 2000). Fe is particularly unavailable for plant uptake in calcareous soils because the alkaline conditions render the Fe less soluble (Masalha et al., 2000). Certain bacteria produce siderophores, which chelate Fe, making it more soluble. There is some controversy about whether plants can use Fe that has been chelated by bacterial siderophores and whether one of the mechanisms by which PGPB improve plant nutrition is through the release of siderophores. Masalha et al. (2000) showed that maize and sunflower have better Fe uptake in nonsterile calcareous soils than in their sterile counterparts, indicating

that soil microorganisms have beneficial effects on Fe uptake. Similarly, Sharma et al. (2003) showed that a *Pseudomonas* sp. increases Fe uptake and reduces chlorosis in mung bean. Both Masalha et al. (2000) and Sharma et al. (2003) attributed the improvement in Fe nutrition to plant uptake of bacterial siderophores. Vansuyt et al. (2007) found that siderophores produced by *Pseudomonas fluorescens* are easily absorbed by *Arabidopsis thaliana* roots. However, Vessey (2003) called into question the efficiency with which plants absorb bacterium–siderophore complexes, and suggested that the main benefit to plants of siderophore-producing bacteria may be that they compete with pathogens for scarce Fe resources. Interestingly, Duijff et al. (1994) concluded that siderophores produced by the PGPB *Pseudomonas putida* contribute significantly to barley Fe nutrition in conditions under which the natural phytosiderophores produced by the plant are decomposed too quickly by the rhizobacteria to be useful in plant Fe uptake.

5.2.4 Some PGPB Induce Changes in Root Morphology

PGPB also enhance plant nutrition by affecting root morphology (Vessey, 2003). Sarig et al. (1992) found that inoculation with *Azospirillum brasilense* increases the length and number of adventitious roots of hydroponically grown sorghum plants. They attributed this change to auxin-like phytohormones produced by *A. brasilense*. López-Bucio et al. (2007) found that inoculation with *Bacillus megaterium* causes an increase in root-hair number and size in *Arabidopsis*; this change could not be attributed to auxins or ethylene and they hypothesized that there is a metabolite produced by the bacteria that may enhance root-hair growth. The same group later found evidence that the effects of *B. megaterium* on *Arabidopsis* roots were caused by cytokinins that were produced by the bacteria (Ortíz-Castro et al., 2008). Bashan and Dubrovsky (1996) analyzed 79 different experiments reported in the literature to determine the changes in shoot-to-root ratio caused by *Azospirillum*, and found a large number of experiments that showed a decrease in the shoot-to-root ratio, although a similar number showed a rise in this parameter. They suggested that *Azospirillum* has an effect on the partitioning of energy and C between the different plant organs, which could explain the nutrition enhancement by *Azospirillum*.

5.2.5 Some PGPB Promote a Symbiotic Relationship between Mycorrhizal Fungi and Roots

One of the most interesting mechanisms by which PGPB enhance plant nutrition involves promotion of the symbiotic relationship between

mycorrhizal fungi and plant roots (Frey-Klett et al., 2007). The PGPB that promote this relationship are called mycorrhiza helper bacteria (MHB) (Frey-Klett et al., 2007). Mycorrhizal fungi infect more than 80% of all terrestrial plants (Giovannetti and Sbrana, 1998), and they contribute significantly to plant nutrition by increasing the absorbing surface of the roots and excreting chelates or enzymes to mobilize insoluble nutrients (Marschner and Dell, 1994). Many PGPB have been found to promote mycorrhizal fungal growth, including *Agrobacterium*, *Streptomyces*, *Pseudomonas*, *Bacillus*, *Paenibacillus*, *Burkholderia*, *Arthrobacter*, *Azospirillum*, *Klebsiella*, *Azospirillum*, *Alcaligenes*, *Rhizobium*, *Bradyrhizobium* and *Brevibacillus* (Frey-Klett et al., 2007). There are six major mechanisms by which MHB promote mycorrhizal fungal growth: (1) stimulating germination of fungal spores (Frey-Klett et al., 2007; Garbaye, 1994; Johansson et al., 2004), (2) promoting mycelial growth (Frey-Klett et al., 2007), (3) removing toxins from the soil that inhibit mycorrhizal growth or positively changing the rhizospheric chemistry or environment to encourage mycorrhizal growth (Frey-Klett et al., 2007; Garbaye, 1994; Johansson et al., 2004), (4) enhancing root receptivity to mycorrhizal infection (Frey-Klett et al., 2007; Garbaye, 1994; Johansson et al., 2004), (5) promoting root branching through hormonal action (Frey-Klett et al., 2007), and (6) increasing the availability of nutrients such as N and P, thus promoting synergy between the mycorrhizal fungi and the plant, both of which require these nutrients (Garbaye, 1994; Johansson et al., 2004).



6. CONCLUSIONS AND FUTURE DIRECTIONS

Biostimulants have been shown to increase nutrient uptake under certain conditions, suggesting their usefulness in reducing fertilizer use without negatively affecting yield. Various mechanisms for these increases have been proposed, and they are summarized in Table 5. However, despite the fact that biostimulants are readily available in the marketplace, they have thus far not significantly reduced fertilizer use in conventional agriculture. Before biostimulants can be effective in reducing fertilizer use, it is important to find the most promising ones for specific conditions, and to research how they are best applied.

Amendment with HS seems promising for improving N uptake. There is a large body of evidence of their effect on both N uptake and N assimilation through stimulation of $H^+ATPase$ and NO_3^- -assimilation enzymes, including some progress into exactly which fraction of the HS is active.

Table 5 Literature suggesting different mechanisms for biostimulants

Type of biostimulant	Improving soil structure or soil microbial activity	Chelation or solubilization of nutrients	Increase H ⁺ ATPase activity	Increase activity of NO ₃ -assimilation Enzymes	Improve activity of arbuscular mycorrhizal fungi	Changes in root morphology
Humic substances	Piccolo et al. (1997)	Chen et al. (2004); Cesco et al. (2000); Pinton et al. (1999b); Sánchez-Sánchez et al. (2006); Sánchez-Sánchez et al. (2005); Sánchez-Sánchez et al. (2002)	Pinton et al. (1999a); Canellas et al. (2008); Zandonadi et al. (2010); Quaggiotti et al. (2004)	Albuzio et al. (1986); Muscolo et al. (1999); Vaccaro et al. (2009)		Malik and Azam (1985)
Amino acids	García-Martínez et al. (2010)	Jie et al. (2008); Ghasemi et al. (2012); Koksal et al. (1999); Rodríguez-Lucena et al. (2010)		Schiavon et al. (2008); Ertani et al. (2009); Maini (2006)		Walch-Liu et al. (2006)

Seaweed extract	Khan et al. (2009)	Spinelli et al. (2010)	Kuwada et al. (2006)	Nelson and van Staden (1984); Steveni et al. (1992); Featonby-Smith and van Staden (1983)
Plant-growth-promoting bacteria		Vessey (2003); Richardson (2001); Gamalero and Glick (2011); Masalha et al. (2000); Sharma et al. (2003); Vansuyt et al. (2007); Duijff et al. (1994)	Frey-Klett et al. (2007); Garbaye (1994); Johansson et al. (2004)	Vessey (2003); Sarig et al. (1992); Ortíz-Castro et al. (2008); López-Bucio et al. (2007); Bashan and Dubrovsky (1996)

One confounding factor is that HS are usually already present in soils, making it difficult to observe positive effects of added HS. More research into the active fraction may help determine when the addition of HS is useful, and how and when it is best applied for positive results.

AA may also be able to decrease the use of N fertilizer by stimulating NO_3 assimilation enzymes, but they probably work most effectively as chelators. They could be best used for correcting micronutrient deficiencies when applied either through foliar spray or as a soil drench with micronutrient fertilizer. There is evidence that AA can be used to chelate micronutrients, but the efficacies of different AA formulations need to be compared, to each other, and to other common chelates such as ethylenediaminetetraacetic acid (EDTA), diethylene triamine pentaacetic acid (DTPA), and EDDHA, under different types of applications (root drench or leaf spray) and under various conditions (high or low pH levels, field, or greenhouse).

A large number of studies have shown that SE can increase root size or root-to-shoot ratio through hormonal action. However, evidence of SE increasing nutrient uptake is still relatively patchy. Only one field study (Rathore et al., 2009) has shown an increase in nutrient uptake due to application of SE, and greenhouse studies do not show consistent results. For example, upon application of SE, an increase in leaf N was observed by Turan and Köse (2004), whereas a decrease in leaf N was observed by Nelson and van Staden (1984); it should be noted, however, that these studies were performed with different types of SE. Other benefits of SE application, such as increased growth, enhanced chlorophyll levels, increased flowering and yield, increased seed germination, enhanced propagation, and increased resistance to pests and pathogens, although not detailed in this review, may offer more fruitful avenues of research. The use of SE as an alternative, environmentally friendly Fe chelate may also hold some promise (Spinelli et al., 2010).

PGPB seem promising for increasing the availability of P and micronutrients in the soil, and enhancing the root system and the arbuscular mycorrhizal fungal network. However, there are technical difficulties in ensuring the survival of the inoculum in the field, and further research is needed on inoculation methods of the most promising PGPB.

Biostimulants hold much promise for reducing fertilizer use, but the literature is often fragmented and unconvincing. Authors tend to look at different nutrients and hypothesize numerous mechanisms without demonstrating any of them consistently. If the use of biostimulants is to reduce that of fertilizers, it is important to elucidate the specific mechanisms in the most

promising substances. Then the active ingredient in the substance should be isolated, and examined in small-scale laboratory experiments, then larger greenhouse experiments and finally, field experiments. The results should consistently show the same mechanisms at work and demonstrate a reduced need for fertilizer use while resulting in equal or greater yield. Without this type of systematic work, it is unlikely that biostimulants will enable a reduction in global fertilizer use.

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