Role of Biostimulants in Horticulture : A Review

Abstract:

In recent days, several technological innovations were proposed in order to improve the production and sustainability through a significant reduction in use of agrochemicals. One 0f the best approach to increase crop productivity is the utilisation of environment-friendly organic products such as "Biostimulants". There are different types of substances that act as biostimulants namely humic and fulvic acid, protein hydrolysates and other N-containing compounds, Seaweed extract and botanicals, Chitosan and other biopolymers, Inorganic compounds, beneficial fungi and bacteria. These biostimulants plays an important role in improving the plant growth, yield and quality of the product. The guava fruits coated with chitosan 1% and stored at 12°C had shown higher firmness, TSS, titratable acidity and maintained greenness with a slow increase in yellow colour by the end of storage. Knowledge on effective use of these biostimulants by understanding their properties has become a challenge to the researchers to improve yield, quality and shelf life of different horticultural products.

Key Words: Biostimulants, Horticulture, humic and fulvic acid

Introduction:

The term "biostimulants" was coined by Zhang and Schmidt, (1997). The 2018 Farm Bill (AI Act) describes Biostimulant as "a substance or microorganism, when applied to seeds, plants, or on the rhizosphere, stimulates natural processes to enhance or benefit nutrient uptake, nutrient use efficiency, tolerance to abiotic stress, or crop quality and yield.They come with tag lines such as "Biological Plant Activator," "Plant Health Stimulator," and "Probiotic for Plants."

In recent days, several technological innovations were proposed to improve the production and sustainability through a significant reduction in use of agrochemicals. But, in an effort to adjust to the exponential trends of our population growth without compromising the integrity of the environment, it is necessary to have a global transition towards sustainable farming. One among the best approach to increase crop productivity is the utilisation of environment-friendly organic products such as "Biostimulants". "Biostimulants are defined as 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" (du Jardin, 2015).

Biostimulants in Horticulture:

"The sustainability in horticultural production is essential to meet the consumer demands which is best achieved by increasing the efficient use of resources to produce healthy products. The most promising practice would be the use of compounds or microorganisms which can enhance plant growth, increase tolerance to unfavourable soil and environmental conditions, and also improve the resource use efficiency". (Brede, 2000) For the past few years, plant biostimulants were considered to be "snake oils—products of mysterious origin that promise to do almost unbelievable miracles". "Unfortunately, initial results of biostimulant application were not acceptable because many of these substances were produced without a scientific base for quality control, but exclusively for marketing purposes" (Brede, 2000). "Though, the improvement in the quality of plant biostimulants and the advancement in the understanding of the biological mechanisms, made biostimulant applications more beneficial". (du Jardin, 2015) European Biostimulants Industry Council (EBIC) defined plant biostimulants as follows: "Plant biostimulant means a material which contains substance(s) and/or microorganisms whose function when applied to plants or the rhizosphere is to stimulate natural processes to benefit nutrient uptake, nutrient efficiency, tolerance to abiotic stress, and/or crop quality, independently of its nutrient content. Biostimulants have no direct action against pests, and therefore they do not fall within the regulatory framework of pesticides" (du Jardin, 2015).

Fig. No. 1 Need for application of biostimulants in plants

Types of Biostimulants:

"The different categories of substances that acts as biostimulants were identified in recent years: (1) humic and fulvic acid, (2) protein hydrolysates and other N-containing compounds (3) Seaweed extract and botanicals (4) Chitosan and other biopolymers (5) Inorganic compounds (6) Beneficial fungi and (7) Beneficial bacteria" (Rose *et al.*, 2014). The nature and importance of each biostimulant is discussed here in this review as follows.

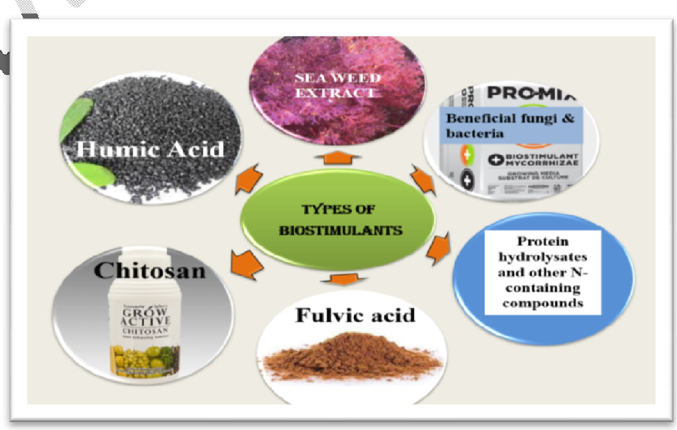

Fig. No. 2 Major categories of biostimulants

1. Humic and fulvic acid:

“Humic substances (HS) are natural constituents of the soil organic matter which is resulting from the decomposition of plant, animal and microbial residues, but also from the metabolic activity of soil microbes. HS are heterogeneous compounds, according to their molecular weight and solubility they are categorised into humin, humic acid and fulvic acid. These compounds also shows complex properties of association/dissociation into supra-molecular colloids, and are influenced by plant roots via the release of protons and exudates” (Colla *et al*., 2015). “Humic substances and their complexes in the soil which result from the interaction between the organic matter, microbes and plant roots. The application of humic substances for promoting plant growth and crop yield needs to optimize these interactions to achieve the expected outputs. This explains why the application of humic substances – soluble humic and fulvic acids fractions – shows inconsistent, but still globally positive, results on plant growth. A recent random-effect meta-analysis of HS applied to plants conducted by Rose *et al*.,(2014), concluded that an overall dry weight increases by 22 ± 4% for shoots and by 21 ± 6% for roots with the application of humic substance”.

“Humic substances have been recognized for the past few decades as essential contributors to soil fertility, acting on physical, physico-chemical, chemical and biological properties of the soil. But, the most bio-stimulant effects of HS refer to the amelioration of root nutrition, via different mechanisms. One among them is the increased uptake of macro and micronutrients, due to the increased cation exchange capacity of the soil containing the polyanionic HS, and to the increased availability of phosphorus by HS interfering with calcium phosphate precipitation. Another important contribution of HS to root nutrition is the stimulation of plasma membrane H^+-ATPases, which convert the free energy released by ATP hydrolysis into a transmembrane electrochemical potential used for the import of nitrate and other nutrient elements. Besides nutrient uptake, proton pumping by plasma membrane ATPases also contributes to cell wall loosening, cell enlargement simultaneously organ growth” (Jindo *et al*., 2012). “HS seem to enhance respiration and invertase activities providing carbon substrates. Hormonal effects are also described, but whether HS contain functional groups recognized by the reception/signalling complexes of plant hormonal pathways, liberate entrapped hormonal compounds, or stimulate hormone-producing microorganisms is often unclear” (du Jardin, 2012). “The bio stimulation activity of HS also refers to stress protection, since, the Phenylpropanoid metabolism is central to the production of phenolic compounds, involved in secondary metabolism and in a wide range of stress responses” (Schiavon *et al*., 2010).

The results from Table 1 indicates that, all treatments with humic acid and amino acid alone or in combinations treatments increased all vegetative growth parameters of banana under study in both seasons. Meanwhile, soil applied with humic acid and amino acid combination increased leaf (N) and (K) content in both seasons. In addition, the treatment of soil applied with l.0g /L humic acid +lg/L amino acid gave the highest values for the above yield and Bunch weight of banana cv. Grand Nine. However, fruit quality (physical and chemical characteristics) was significantly improved by soil applied with different humic and amino acid treatments either alone or in combinations.

Table 1: Effect of soil application with humic acid and amino acid on physical characteristics of bunch and fingers of banana cv. Grand Naine

Treatment	Bunch weight (kg)	No. of hands/ bunch	Hand weight (kg)	No. of fingers/ hand	Finger weight (g)	Finger length (cm)
T_1 – Control (recommended doses)	14.18	10.00	1.42	15.00	94.67	14.30
T_2 – Humic acid 5 g/l	16.38	11.00	1.49	15.67	95.00	15.06
T_3 - Humic acid 10 g/l	18.18	11.33	1.60	16.67	96.33	15.82
T_4 - Amino acid 0.5 g/l	19.81	12.00	1.65	17.00	97.00	16.35
T_5 - Amino acid 1.0g/l	22.39	12.67	1.77	17.33	102.00	16.75
T_6 - Humic acid 5 g/l + Amino acid 0.5 g/l	25.57	13.00	1.96	18.33	107.00	17.42
T_7 - Humic acid 5 g/l + Amino acid 1.0 g/l	25.50	12.67	2.01	18.33	109.67	18.15
T_8 - Humic acid 10 g/l + Amino acid 0.5 g/l	29.11	13.33	2.18	18.67	117.00	18.17
T_9 - Humic acid 10 g/l + Amino acid 1.0 g/l	31.20	14.00	2.23	19.00	117.33	18.30

Note: They are added into four doses during the 1st week of April to July.

The mean was compared by using the method of least significant differences (LSD at 0.05)

From Table 2 it is concluded that, the doses of humic substances influenced only pseudostem height. Hand yield, as well as fruit length and diameter of 'BRS Princesa' banana was higher in plants fertigated with humic substance and plant extract in comparison with plants fertigated with only humic substance.

Table 2: Effect of humic substance on yield of banana cv. BRS Princesa

HS dose (L/ha)	No. of fruits/ hand	No. of hands/ bunch	Hand yield (t/ha)	Bunch yield (t/ha)	Fruit diameter (cm)
0.0	97.90	6.07	25.52	29.10	36.03
21.14	**103.25**	**6.57**	**27.83**	**31.75**	**36.90**
31.71	101.50	6.47	27.27	30.87	36.25
42.28	102.40	6.50	27.44	30.93	35.20

63.42	99.60	6.32	25.53	29.11	36.23
CV (%)	9.54	5.09	8.47	7.88	5.44

Note: Humic substance (HS) composed by humic acids (200 g/kg), fulvic acids (102 g/kg) and potassium (26.6 g/kg) applied through fertigation

2. Protein hydrolysates and other N-containing compounds:

"Amino-acids and peptide mixtures are obtained by chemical and enzymatic protein hydrolysis from agro-industrial by-products, from both plant sources (crop residues) and animal wastes (e.g. collagen, epithelial tissues)", (du Jardin, 2012; Calvo *et al*., 2014; Halpern *et al*., 2015). "Chemical synthesis can also be used for single or mixed compounds. Other nitrogenous molecules include betaines, polyamines and 'non-protein amino acids', which are diversified in higher plants but poorly characterized with regard to their physiological and ecological roles" (Vranova *et al*., 2011). "Glycine betaine is a special amino acid derivative with well-known anti-stress properties" (Chen and Murata, 2011). "Case by case, these compounds have been shown to play multiple roles as bio stimulants of plant growth" (Calvo *et al*., 2014; du Jardin, 2012, Halpern *et al*., 2015).

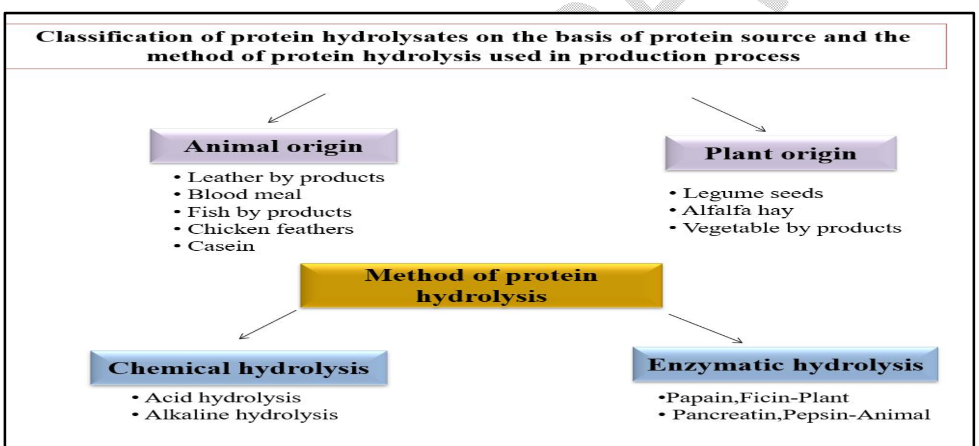

Fig. No. 3 Classification and method of preparation protein hydrolysates

"Direct effects of these compounds on plants include modulation of N uptake and assimilation, by the regulation of enzymes involved in N assimilation and of their structural genes, and by acting on the signalling pathway of N acquisition in roots. By regulating enzymes of the TCA cycle, they also contribute to the cross talk between C and N metabolisms. Hormonal activities are also reported in complex protein and tissue hydrolysates. Chelating effects are reported for some amino acids (like proline) which may protect plants against heavy metal and other abiotic stresses but also contribute to micronutrients mobility and acquisition. Antioxidant activity is conferred by the scavenging of free radicals by some of the nitrogenous compounds, including glycine betaine, glutathione and proline, which contributes to the mitigation of environmental stress" (Colla *et al*., 2014).

3. Seaweed extracts and botanicals:

"The use of fresh seaweeds as source of organic matter and as fertiliser is an age old practice in agriculture, but bio stimulant effects have been recorded only recently. This prompts the commercial use of seaweed extracts and its purified compounds, which include the polysaccharides laminarin, alginates and carrageenan's and their breakdown products. Other constituents contributing to the plant growth promotion include micro and macronutrients, sterols, N- containing compounds like betaines, and hormones" (Craigie, 2011; Khan *et al*., 2009). "Several of these compounds are indeed unique to their algal source, explaining the increasing interest of the scientific community and of the industry for these taxonomic groups. Most of the algal species belong to the phylum of brown algae such as *Ascophyllum, Fucus, Laminaria* as main genera-, but carrageenan's originate from red seaweeds, which correspond to a distinct phylogenetic line. Product names of more than 20 seaweed extracts used as plant growth bio stimulant have been listed" by Khan *et al.* (2009).

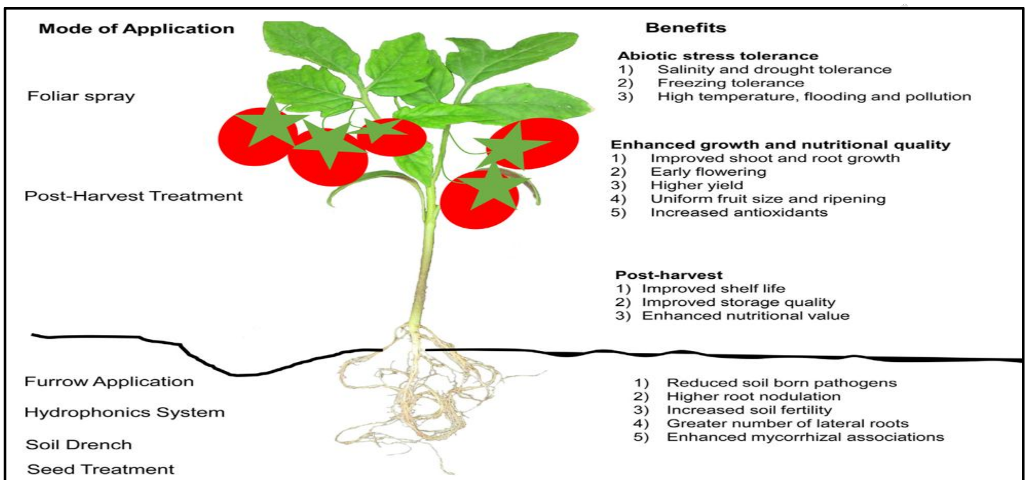

Fig. No. 4 Methods of application of seaweed extract and its effect on plant growth

"Seaweed extracts act both on soils as well as on plants. They can be applied to soils or in hydroponic solutions or as foliar treatments. In soils, the polysaccharides present in seaweed extracts contribute to gel formation, water retention and soil aeration. Similarly, polyanionic compounds contribute to the fixation and exchange of cations, which is also of interest for the fixation of heavy metals and for soil remediation. Positive effects via the soil microflora are also described, with the promotion of plant growth-promoting bacteria and pathogen antagonists in suppressive soils. In plants, nutritional effects via the provision of micro- and macronutrients indicate that they act as fertilisers, beside their other roles. Impacts on seed germination, plant establishment and on further growth and development is associated with hormonal effects, which is viewed as major causes of bio stimulation activity on crop plants" (Craigie *et al*., 2008; Craigie, 2011; Khan *et al*., 2009).

"Although cytokinin's, auxins, abscisic acid, gibberellins and other classes of hormone-like compounds such as sterols and polyamines, have been identified in seaweed extracts by bioassays and by immunological tools" (Craigie, 2011). "There is evidence that, the hormonal effects of extracts of the brown seaweed (*Ascophyllum nodosum*) are explained to a large extent by the down- and upregulation of hormone biosynthetic genes in plant tissues, and to a lesser extent to the hormonal contents of the seaweed extracts themselves Molecular genetics, i.e. hormone mutants in Arabidopsis and transcript analysis by RT-qPCR, were used to reach this conclusion" (Wally *et al*., 2013).

4. Chitosan and other biopolymers:

"Chitosan is a deacetylated form of the biopolymer chitin, produced naturally and industrially. Poly- and oligomers of variable, controlled sizes are used in the food, cosmetic, medical and agricultural sectors" (Krishna and Rao, 2017). "The physiological effects of chitosan oligomers in plants are the results of the capacity of its polycationic compound to bind a wide range of cellular components, including DNA, plasma membrane and cell wall constituents, but also to bind specific receptors involved in defence gene activation, in a similar way as plant defence elicitors" (El Hadrami *et al.*, 2010; Hadwiger, 2013; Katiyar *et al.*, 2015; Yin *et al.*, 2010).

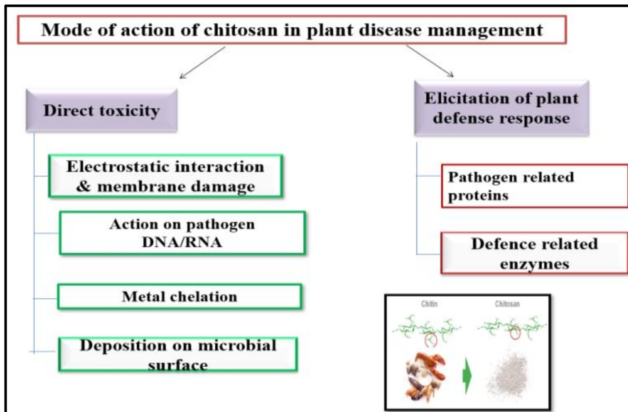

Fig. No. 5 Mode of action of Chitosan in plants

"Chitin and chitosan apparently use distinct receptors and signalling pathways. Among the cellular consequences of the binding of chitosan to more or less specific cell receptors, hydrogen peroxide accumulation and Ca^{2+} leakage into the cell have been demonstrated, which are expected to cause large physiological changes, as these are key players in the signalling of stress responses and in the development regulation. Analysis of the proteome (Ferri *et al*., 2014) or transcriptome (Povero *et al.*, 2011) of plant tissues treated with chitosan confirming this assumption". "In consequence, agricultural applications of chitosan have been developed over the years, focusing on plant protection against fungal pathogens, but broader agricultural uses bear on tolerance to abiotic stress (drought, salinity, cold stress) and quality traits related to primary and secondary metabolisms. Stomatal closure induced by chitosan via an ABA-dependent mechanism participates to the environmental stress protection" (Iriti *et al.*, 2009).

"Several poly and oligomers of biological origin or (hemi-) synthetic variants are increasingly used in agriculture as elicitors of plant defence, including seaweed polysaccharides. A good example is laminarin, which is a storage glucan of brown algae is used in agricultural applications. Although a distinction has to be made between biocontrol and bio stimulation (e.g. enhancing abiotic stress), signalling pathways may be inter connected and both effects may practically result from the application of the same inducers" (Gozzo and Faoro, 2013).

5. Inorganic compounds:

"Chemical elements that, promote plant growth and essential to particular group of plants but are not required by all plants are called beneficial elements. The five main beneficial elements are Al, Co, Na, Se and Si, present in soils and in plants as different inorganic compounds and as insoluble forms like amorphous silica (SiO2 and H_2O) in gramineous species. These beneficial functions can be constitutive, like the strengthening of cell walls by silica deposits, or expressed in defined environmental conditions, like pathogen attack for selenium and osmotic stress for sodium. Definition of beneficial elements is thus not limited to their chemical natures, but must also refer to the special contexts where the positive effects on plant growth and stress response may be observed. It may be assumed that the bioactivity of some complex bio stimulants, like extracts of seaweeds, of crop residues or animal wastes, involves the physiological functions of the contained beneficial elements" (Pilon-Smits *et al.*, 2009).

"Many effects of inorganic compounds are reported by the scientific literature, which promote plant growth, the quality of plant products and tolerance to abiotic stress. This includes cell wall rigidification, osmoregulation, reduced transpiration by crystal deposits, thermal regulation via radiation reflection, enzyme activity by co-factors, plant nutrition via interactions with other elements during uptake and mobility, antioxidant protection, interactions with symbionts, pathogen and herbivore response, protection against heavy metals toxicity, plant hormone synthesis and signalling. Inorganic salts of beneficial and essential elements – chlorides, phosphates, phosphites, silicates and carbonates – have been used as fungicides. Although the mode of action is not yet fully established, however, these inorganic compounds influence osmotic, pH and redox homeostasis, hormone signalling and enzymes involved in stress response (e.g. peroxidases). Their function as bio-stimulant of plant growth, acting on nutrition efficiency and abiotic stress tolerance, hence, distinct from their fungicidal action and from their fertilizer function as sources of nutrients, deserves more attention" (Deliopoulos *et al.*, 2010).

6. Beneficial fungi:

"Fungi interact with plant roots in different ways, from mutualistic symbiosis (i.e. when both organisms live in direct contact with each other and establish mutually beneficial relationships) to parasitism" (Behie and Bidochka, 2014). "Plants and fungi have co-evolved since, the origin of terrestrial plants and the concept of mutualism – parasitism continuum is useful to describe the extended range of relationships that developed over the evolutionary times" (Bonfante and Genre, 2010). "Mycorrhizal fungi are a heterogeneous group of fungi which establish symbiosis with over 90 % of all plant species" (Graham *et al*, 2013).

"Among the different forms of physical interactions involved, the Arbuscule-Forming Mycorrhiza (AMF) are a widespread type of endomycorrhiza associated with crop and horticultural plants, where fungal hyphae of Glomeromycota species penetrate root cortical cells and form branched structures called arbuscules" (Bonfante and Genre, 2010; Behie and Bidochka, 2014). "There is an increasing interest for the use of mycorrhiza to promote sustainable horticulture, considering the widely accepted benefits of the symbioses to nutrition efficiency (for both macronutrients, especially P, and micronutrients), water balance, biotic and abiotic stress protection of plants" (Augé, 2001; Gianinazzi *et al.*, 2010; Hamel and Plenchette, 2007; Harrier and Watson, 2004).

"Recent knowledge also points to the existence of hyphal networks which interconnect not only fungal and plant partners but also individual plants within a plant community. This could have significant ecological and horticultural implications since, there is evidence that, the fungal conduits allow for interplant signalling (Johnson and Gilbert, 2015). As a further area of research, AMF form tripartite associations with plants and rhizobacteria which are relevant in practical field situations" (Simard *et al.*, 2012).

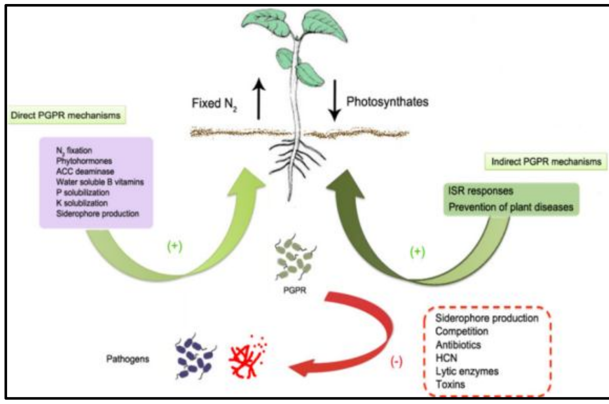

Fig. No. 6 Mode of action of PGPR in plants

"Fungal-based products applied to plants to promote nutrition efficiency, tolerance to stress, crop yield and product quality should fall under the concept of bio-stimulants. Major limitations on their use are the technical difficulty to propagate AMF on a large scale, due to their biotrophic character (Dalpé and Monreal, 2004), and, more fundamentally, the lack of understanding of the determinants of the host specificities and population dynamics of mycorrhizal communities in agroecosystems". "Nevertheless, other fungal endophytes, like *Trichoderma* spp. (Ascomycota) and Sebacinales (Basidiomycota, with *Piriformospora indica* as model organism), which are distinct from the mycorrhizal species, are able to live at least part of their life cycle away from the plant, to colonize roots and to transfer nutrients to their hosts, using poorly understood mechanisms" (Behie and Bidochka, 2014). "They are also receiving increasing attention, both as plant inoculants easier to multiply in vitro and as model organisms for dissecting the mechanisms of nutrient transfer between fungal endosymbionts and their hosts. Some of these fungi, mainly Trichoderma spp., have been extensively studied and used for their bio pesticidal (mycoparasitic) and biocontrol (inducer of disease resistance) capacities, and have been exploited as sources of enzymes by biotechnological industries" (Mukherjee *et al.*, 2012). "There is convincing evidence that, many plant responses are also induced, including increased tolerance to abiotic stress, nutrient use efficiency and organ growth and morphogenesis" (Colla *et al*., 2014). On the basis of these effects, these fungal endophytes may be regarded as bio stimulants, though their uses are currently supported by claims as biopesticides.

7. Beneficial bacteria:

"Bacteria interact with plants in all possible ways (Ahmad *et al*., 2008): (i) as for fungi there is a continuum between mutualism and parasitism; (ii) bacterial niches extend from the soil to the interior of cells, with intermediate locations called the rhizosphere and the rhizoplane; (iii) associations may be transient or permanent, some bacteria being even vertically transmitted via the seed; (iv) functions influencing plant life cover participation to the biogeochemical cycles, supply of nutrients, increase in nutrient use efficiency, induction of disease resistance, enhancement of abiotic stress tolerance, modulation of morphogenesis by plant growth regulators". "With regard to the agricultural uses of bio stimulants, two main types should be considered within this taxonomic, functional and ecological diversity: (i) mutualistic endosymbionts of the type Rhizobium and (ii) mutualistic, rhizospheric PGPRs ('plant growth-promoting rhizobacteria'). Rhizobium and related taxa are commercialised as biofertilizers, i.e. microbial inoculants facilitating nutrients acquisition by plants" (Gaiero *et al.*, 2013).

" PGPRs are multifunctional and influence all aspects of plant life: nutrition and growth, morphogenesis and development, response to biotic and abiotic stress, interactions with other organisms in the agroecosystems" (Ahmad *et al*., 2008; Babalola, 2010; Berendsen *et al*., 2012; Berg *et al*., 2014). "Several of these functions are generally fulfilled by the same organisms, some are strain-specific and others are dependent on synergisms within bacterial consortia. Agricultural uses of PGPRs are constrained by this complexity, by the variable responses of the plant cultivars and the receiving environments also the technical difficulties associated with the formulation of the inoculants" (Bhattacharyya and Jha, 2012).

Conclusions:

The following forces should steer the future of plant biostimulants. We now understand plant physiology better than ever before, thanks to scientific and technological developments in numerous areas over the previous few decades. The majority of these accomplishments have been made using a small number of model organisms in controlled conditions. The challenge now is to apply this information and these technologies to characterize biostimulants and their effects on a diverse variety of cultivated plants. High-throughput plant phenotyping platforms, for example, have been developed to characterize mutants generated in functional genomics studies, but they should inspire research into the modes of action of biostimulants and their interactions with environmental stressors and plant genotypes.

Biostimulant use in agriculture and horticulture will necessitate locally and temporally tailored solutions. Monitoring methods for biostimulant efficacy will be required, as will stewardship programs for optimizing their use. Long-term implications, such as ecological services and biogeochemical cycles, should be examined and incorporated into farm decision-making. Stakeholders, farmers, public research, and regulatory organizations will all need to be involved in order to gain the benefits that biostimulants can bring to lucrative and sustainable plant production.

Conference disclaimer:

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