Review Article

Role of Biostimulants in Horticulture

Abstract:

In recent years, several technical and technological innovations were proposed in order to improve the sustainability of production systems through a significant reduction of agrochemicals. One such best approach to increase crop productivity is the development of environment-friendly organic products named “Biostimulants” (Du Jardin, 2015). There are different categories of substances that act as biostimulants : (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 effect of chitosan coated on postharvest quality of guava cv. Allahabad Safeda fruits were studied at room temperature by treating with chitosan (1 and 2%), acetic acid 1% or untreated and results revealed that chitosan 1% treated fruits 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. (Krishna and Rao, 2017). A challenge is now to use this knowledge and these tools for the characterisation of biostimulants and their effects on a wide range of fruit crops to improve fruit yield.

Introduction:

The term “biostimulants” was proposed by Zhang and Schmidt, 1997. 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 years, several technical and technological innovations were proposed in order to improve the sustainability of production systems through a significant reduction 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 such best approach to increase crop productivity is the development of environment-friendly organic products named “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 of horticultural production is essential to meet consumer demands. This is best achieved by increasing the efficient use of resources to make and provide healthy products. A promising practice would be the use of substances and/or microorganisms that enhance plant growth, increase tolerance to unfavourable soil and environmental conditions, and also improve the resource use efficiency. For 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 gratifying because many of these substances were produced without a scientific base for development or quality control, but exclusively for marketing purposes. However, the improvement in the quality of plant biostimulants and the advancement of the understanding of the biological mechanisms, made biostimulant applications really beneficial. 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).

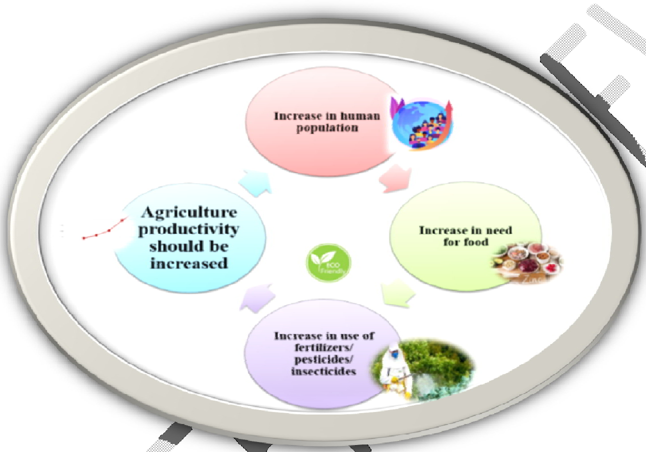

Fig. No. 1 Need for application of biostimulants in plants

Types of Biostimulants:

There are different types of biostimulants namely humic and fulvic acids which occur naturally in soils as a result of breakdown of organic matter, protein hydrolysates which include small peptides and amino acids, they are building blocks of all proteins, seaweed plant extracts containing minor nutrients and plant hormones such as cytokinins. The extracts contain substances that can stimulate plant metabolism or natural defence systems, chitosan and other biopolymers derived from the shells of crustaceans, inorganic compounds which are mineral-based molecules such as phosphites and minor elements such as silicon and lastly beneficial microbes and their mode of action of these products is based on the activity of live fungi and/or bacteria (du Jardin, 2012).

A comprehensive review of international scientific literature on plant biostimulants was recently conducted by Patrick du Jardin in the frame of a contract signed with European Commission (du Jardin, 2012). 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). However, the former bibliographic analysis did not include the bio-stimulant effects of beneficial microorganisms (i.e., arbuscular mycorrhizal fungi, other beneficial fungi like Trichoderma spp., and plant growth-promoting rhizobacteria). The nature and importance of each biostimulant is discussed here in this review as follows.

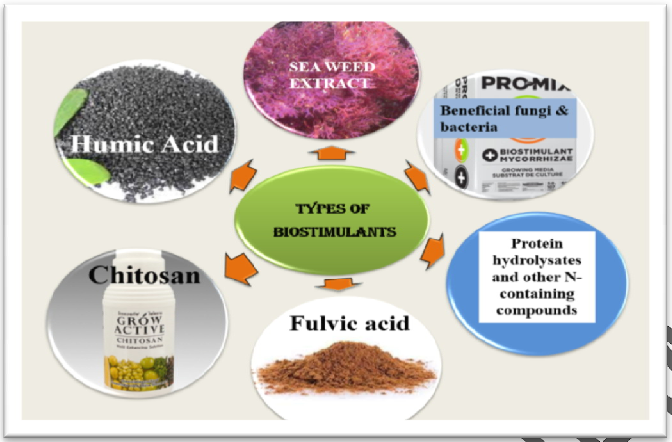

Fig. No. 2 Major categories of biostimulants

1. Humic and fulvic acid:

Humic substances (HS) are natural constituents of the soil organic matter, resulting from the decomposition of plant, animal and microbial residues, but also from the metabolic activity of soil microbes using these substrates. HS are collections of heterogeneous compounds, originally categorized according to their molecular weights and solubility into humins, humic acids and fulvic acids. These compounds also show complex dynamics of association/dissociation into supra-molecular colloids, and this is influenced by plant roots via the release of protons and exudates. Humic substances and their complexes in the soil thus result from the interplay between the organic matter, microbes and plant roots. Any attempt to use 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, yet 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 long as essential contributors to soil fertility, acting on physical, physico-chemical, chemical and biological properties of the soil. Most bio-stimulant effects of HS refer to the amelioration of root nutrition, via different mechanisms. One of 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 nutrients. Besides nutrients uptake, proton pumping by plasma membrane ATPases also contributes to cell wall loosening, cell enlargement and organ growth (Jindo *et al.*, 2012). HS seem to enhance respiration and invertase activities providing C 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 proposed bio stimulation activity of HS also refers to stress protection. Phenylpropanoid metabolism is central to the production of phenolic compounds, involved in secondary metabolism and in a wide range of stress responses. High-molecular mass HS have been shown to enhance the activity of key enzymes of this metabolism in hydroponically-grown maize seedlings, suggesting stress response modulation by HS (Schiavon *et al*., 2010).

The results from Table 1 indicated that, all treatments with humic acid and amino acid alone or in combinations treatments increased all vegetative growth parameter 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 +l g/L amino acid gave the highest values for the above yield and Bunch weight. 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.

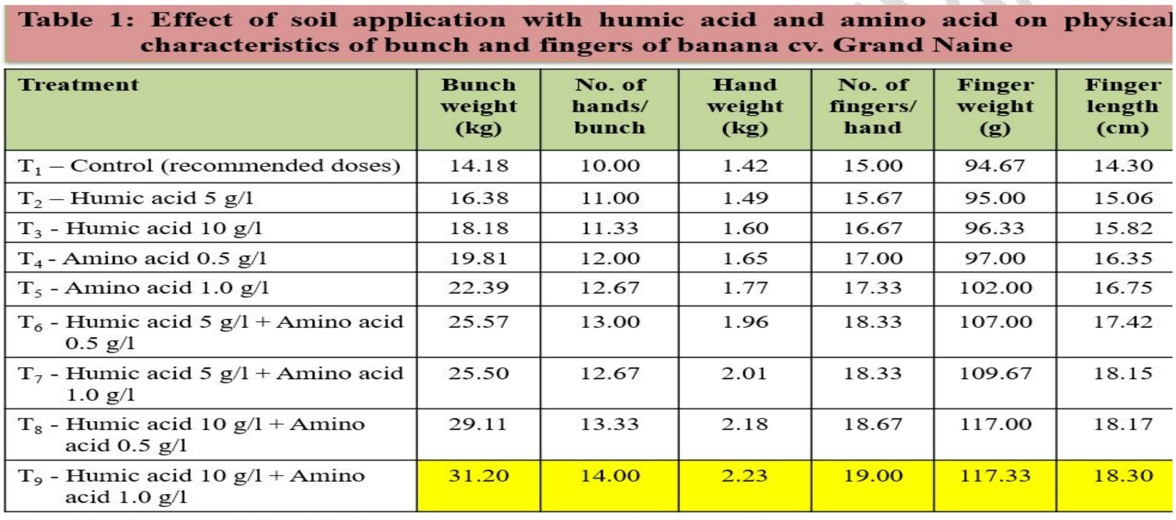

Table 1: Effect of soil application with humic acid and amino acid on physical characteristics of bunch and fingers of banana cv. Grand Naine

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 1.0 g/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 were higher in plants fertigated with humic substance and plant extract in comparison with plants fertigated with only humic substance.

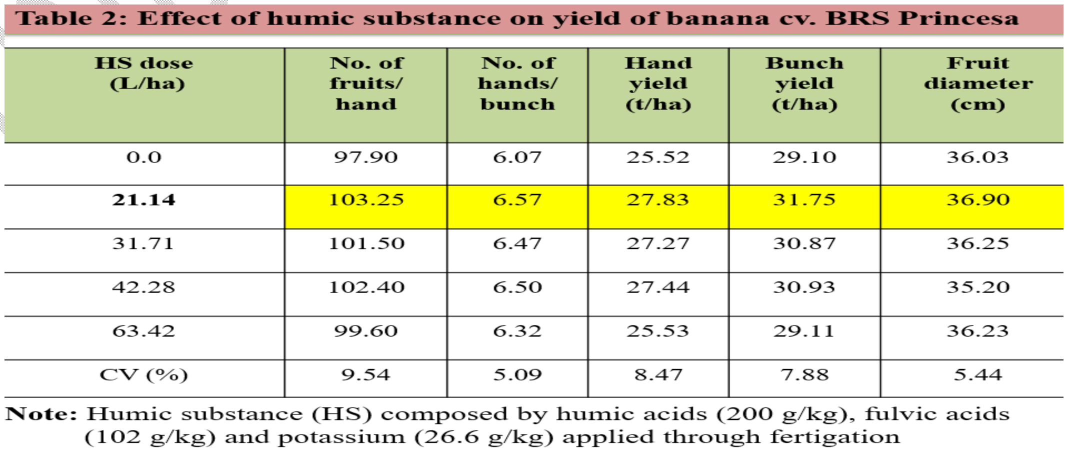

Table 2: Effect of humic substance on yield of banana cv. BRS Princesa

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 peptides 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 case of 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).

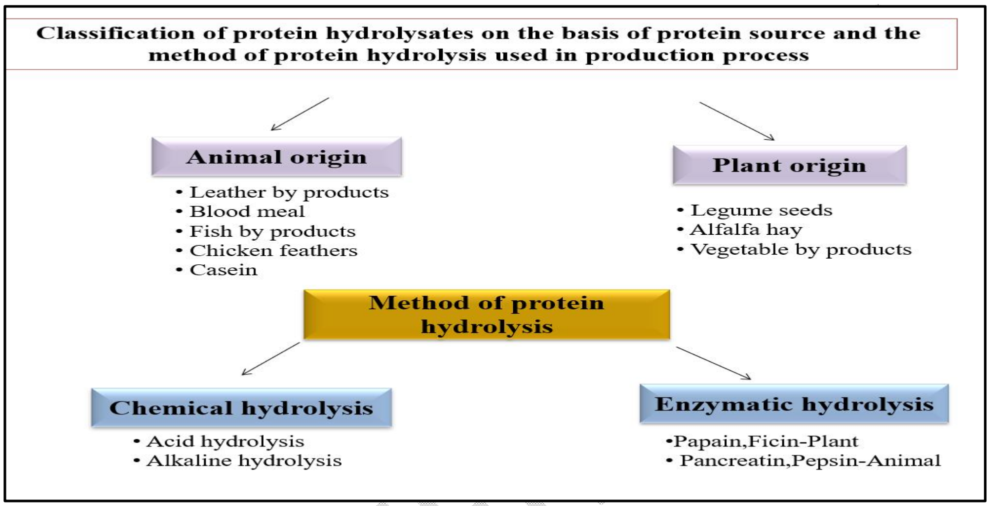

Fig. No. 3 Classification and method of preparation protein hydrolysates

Direct effects 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 metals 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 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 ancient in agriculture, but bio stimulant effects have been recorded only recently. This prompts the commercial use of seaweed extracts and of 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 – with *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 products used as plant growth bio stimulant have been listed by Khan *et al*. (2009).

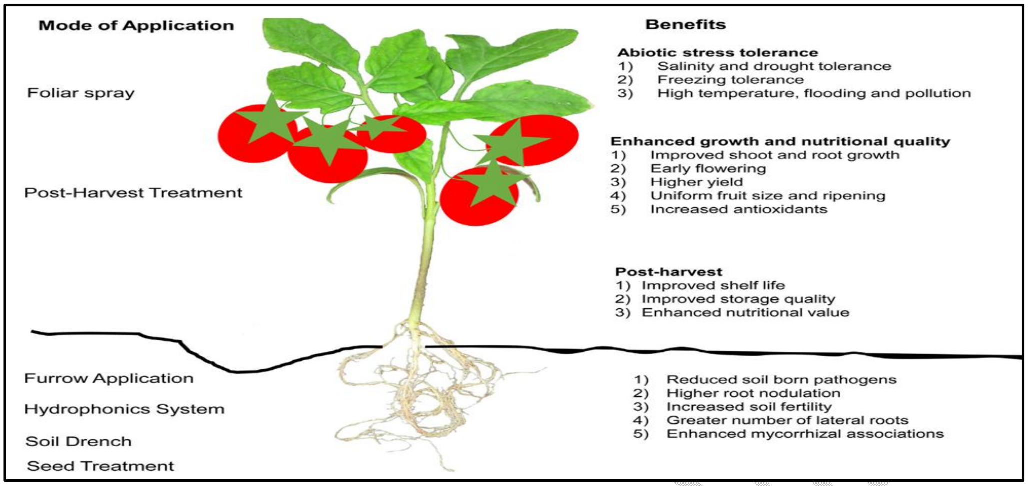

Fig. No. 4 Methods of application of seaweed extract and its effect on plant growth

Seaweeds act both on soils as well as on plants. They can be applied to soils, in hydroponic solutions or as foliar treatments. In soils, their polysaccharides contribute to gel formation, water retention and soil aeration. The 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 and 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, like 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 (Wally *et al*., 2013). Molecular genetics, i.e. hormone mutants in Arabidopsis and transcript analysis by RT-qPCR, were used to reach this conclusion.

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. The physiological effects of chitosan oligomers in plants are the results of the capacity of this 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).

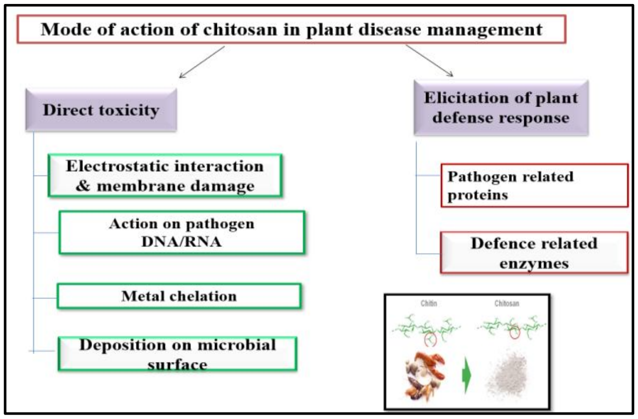

Fig. No. 5 Mode of action of Chitosan in plants

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 confirm 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 conferred by this bio stimulant (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, a storage glucan of brown algae, of which purified preparations are 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 interconnected 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 may be essential to particular taxa 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 salts 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 beneficial elements 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 taxa which establish symbiosis with over 90 % of all plant species (Johnson and Graham, 2013).

Among the different forms of physical interactions and taxa 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 agriculture, 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 agricultural 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).

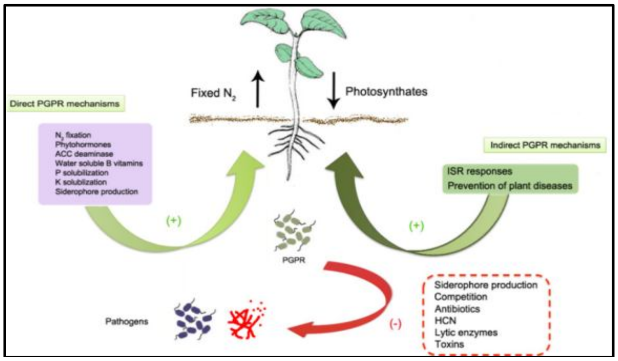

Fig. No. 6 Mode of action of PGPR in plants

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), distinct from the mycorrhizal species, are able to live at least part of their life cycle away from the plant, to colonize roots and, as shown recently, to transfer nutrients to their hosts, using poorly understood mechanisms (Behie and Bidochka, 2014). They are 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*., 2015). On the basis of these effects, these fungal endophytes may be regarded as bio stimulants, though their agricultural 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. The biology and agricultural uses of the Rhizobium-based symbiosis have been extensively reviewed by the scientific literature and in textbooks (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, 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 future of plant biostimulants should be driven by the following lines of force. From the laboratory to the field, we understand the physiology of plants today better than ever, thanks to scientific and technical breakthroughs in many disciplines over the last decades. Most of these achievements have used a limited number of model organisms in controlled environments. A challenge is now to use this knowledge and these tools for the characterisation of biostimulants and their effects on a wide range of cultivated plants. For example, high-throughput plant phenotyping platforms have been developed for the characterisation of mutants produced in functional genomics studies, but they should (and do) inspire studies for understanding the modes of action of biostimulants and their interactions with environmental stressors and with plant genotypes (Aira et al., 2010).

Agricultural and horticultural use of biostimulants will require locally and temporally adapted solutions. Monitoring tools for the efficacy of biostimulants will be needed and stewardship plans optimising their use defined. Longer term effects, via ecological services and biogeochemical cycles, should also be assessed and integrated in the decision-making process on the farm. Involvement of stakeholders, farmers, public research and regulatory bodies will be needed to reap the benefits that biostimulants can bring to profitable and sustainable plant productions. (Aira et al., 2010).

References

Ahmad F, Ahmad I, Khan MS. Screening of free-living rhizospheric bacteria for their multiple plant growth promoting activities. Microbiological research. 2008; Mar 15;163(2):173-81.

Aira M, Gómez-Brandón M, Lazcano C, Bååth E, Domínguez J. Plant genotype strongly modifies the structure and growth of maize rhizosphere microbial communities. Soil Biology and Biochemistry. 2010;42(12):2276-81.

Antonelli A, Ferrari SM, Giuggioli D, Ferrannini E, Ferri C, Fallahi P. Chemokine (C–X–C motif) ligand (CXCL) 10 in autoimmune diseases. Autoimmunity reviews. 2014; Mar 1;13(3):272-80.

Augé RM. Water relations, drought and vesicular-arbuscular mycorrhizal symbiosis. Mycorrhiza. 2001;11(1):3-42.

Babalola OO. Beneficial bacteria of agricultural importance. Biotechnology letters. 2010 Nov;32:1559-70.

Behie SW, Bidochka MJ. Nutrient transfer in plant–fungal symbioses. Trends in plant science. 2014; Nov 1;19(11):734-40.

Berendsen RL, Pieterse CM, Bakker PA. The rhizosphere microbiome and plant health. Trends in plant science. 2012 Aug 1;17(8):478-86.

Berg G, Grube M, Schloter M, Smalla K. Unraveling the plant microbiome: looking back and future perspectives. Frontiers in microbiology. 2014;5.

Bhattacharyya PN, Jha DK. Plant growth-promoting rhizobacteria (PGPR): emergence in agriculture. World Journal of Microbiology and Biotechnology. 2012 Apr;28:1327-50.

Bonfante P, Genre A. Mechanisms underlying beneficial plant–fungus interactions in mycorrhizal symbiosis. Nature communications. 2010; Jul 27;1(1):48.

Cabantous S, Nguyen HB, Pedelacq JD, Koraïchi F, Chaudhary A, Ganguly K, Lockard MA, Favre G, Terwilliger TC, Waldo GS. A new protein-protein interaction sensor based on tripartite split-GFP association. Scientific reports. E2013; Oct 4;3(1):1-9.

Calvo P, Nelson L, Kloepper JW. Agricultural uses of plant biostimulants. Plant and soil. 2014 Oct; 383:3-41.

Canellas LP, Olivares FL, Aguiar NO, Jones DL, Nebbioso A, Mazzei P, Piccolo A. Humic and fulvic acids as biostimulants in horticulture. Scientia horticulturae. 2015 Nov 30;196:15-27.

Chen TH and Murata N. Glycinebetaine protects plants against abiotic stress: mechanisms and biotechnological applications. *Plant, cell & environment*, 2011; *34*(1), pp.1-20.

Colla G, Rouphael Y, Canaguier R, Svecova E, Cardarelli M. Biostimulant action of a plant-derived protein hydrolysate produced through enzymatic hydrolysis. Frontiers in plant science. 2014 Sep 9;5:448.

Craigie JS 2011. Seaweed extract stimuli in plant science and agriculture. *Journal of applied phycology*, 2011; *23*, pp.371-393.

Dalpe Y, Monreal M. Arbuscular mycorrhiza inoculum to support sustainable cropping systems. Crop management. 2004;3(1):1-1.

Dash A, Samant D, Dash DK, Dash SN and Mishra KN, Influence of Ascophyllum nodosum extract, homobrassinolide and triacontanol on fruit retention, yield and quality of mango, Journal of Environmental Biology. 2020; 4(2): 1085-1091.

Deliopoulos T, Kettlewell PS, Hare MC. Fungal disease suppression by inorganic salts: a review. Crop Protection. 2010; Oct 1;29(10):1059-75.

Du Jardin P. Plant biostimulants: Definition, concept, main categories and regulation. Scientia horticulturae. 2015 Nov 30;196:3-14.

El Hadrami A, Adam LR, El Hadrami I, Daayf F. Chitosan in plant protection. Marine drugs. 2010; Mar 30;8(4):968-87.

Ferri M, Franceschetti M, Naldrett MJ, Saalbach G, Tassoni A. Effects of chitosan on the protein profile of grape cell culture subcellular fractions. Electrophoresis. 2014 Jun;35(11):1685-92.

Gaiero JR, McCall CA, Thompson KA, Day NJ, Best AS, Dunfield KE. Inside the root microbiome: bacterial root endophytes and plant growth promotion. American journal of botany. 2013 Sep;100(9):1738-50.

Gianinazzi S, Gollotte A, Binet MN, van Tuinen D, Redecker D, Wipf D. Agroecology: the key role of arbuscular mycorrhizas in ecosystem services. Mycorrhiza. 2010; Nov;20(8):519-30.

Gozzo F, Faoro F. Systemic acquired resistance (50 years after discovery): moving from the lab to the field. Journal of agricultural and food chemistry. 2013; Dec 26;61(51):12473-91.

Hadwiger LA. Multiple effects of chitosan on plant systems: Solid science or hype. Plant science. 2013; Jul 1;208:42-9.

Halpern M, Bar-Tal A, Ofek M, Minz D, Muller T, Yermiyahu U. The use of biostimulants for enhancing nutrient uptake. Advances in agronomy. 2015 Jan 1;130:141-74.

Hamel C, editor. Mycorrhizae in crop production. CRC Press; 2007 Nov 7.

Harrier LA, Watson CA. The potential role of arbuscular mycorrhizal (AM) fungi in the bioprotection of plants against soil-borne pathogens in organic and/or other sustainable farming systems. Pest Management Science: formerly Pesticide Science. 2004 Feb;60(2):149-57.

He C, Hu Y, Yin L, Tang C, Yin C. Effects of particle size and surface charge on cellular uptake and biodistribution of polymeric nanoparticles. Biomaterials. 2010; May 1;31(13):3657-66.

Iriti M, Picchi V, Rossoni M, Gomarasca S, Ludwig N, Gargano M, Faoro F. Chitosan antitranspirant activity is due to abscisic acid-dependent stomatal closure. Environmental and Experimental Botany. 2009 Sep 1;66(3):493-500.

Jindo K, Suto K, Matsumoto K, García C, Sonoki T, Sanchez-Monedero MA. Chemical and biochemical characterisation of biochar-blended composts prepared from poultry manure. Bioresource technology. 2012 Apr 1;110:396-404.

Johnson D, Gilbert L. Interplant signalling through hyphal networks. New Phytologist. 2015; Mar;205(4):1448-53.

Katiyar D, Hemantaranjan A, Singh B. Chitosan as a promising natural compound to enhance potential physiological responses in plant: a review. Indian Journal of Plant Physiology. 2015; Mar;20:1-9.

Khan AA, Jilani G, Akhtar MS, Naqvi SM, Rasheed M. Phosphorus solubilizing bacteria: occurrence, mechanisms and their role in crop production. J agric biol sci. 2009; Jan 1;1(1):48-58.

Krishna KR, Rao DV. Influence of chitosan coating and storage temperatures on postharvest quality of guava. Indian Journal of Horticulture. 2017;74(3):466-70.

Morales-Payan JP, Stall WM. Papaya (Carica papaya) response to foliar treatments with organic complexes of peptides and amino acids. In Proceedings of the Florida State Horticultural Society 2003 Dec 1 (Vol. 116, pp. 30-32).

Mukherjee S, Sushma V, Patra S, Barui AK, Bhadra MP, Sreedhar B, Patra CR. Green chemistry approach for the synthesis and stabilization of biocompatible gold nanoparticles and their potential applications in cancer therapy. Nanotechnology. 2012; Oct 12;23(45):455-103.

Pilon-Smits EA, Quinn CF, Tapken W, Malagoli M, Schiavon M. Physiological functions of beneficial elements. Current opinion in plant biology. 2009; Jun 1;12(3):267-74.

Povero G, Gonzali S, Bassolino L, Mazzucato A, Perata P. Transcriptional analysis in high-anthocyanin tomatoes reveals synergistic effect of Aft and atv genes. Journal of plant physiology. 2011; Feb 15;168(3):270-9.

Rastall RA, Gibson GR. Recent developments in prebiotics to selectively impact beneficial microbes and promote intestinal health. Current Opinion in Biotechnology. 2015; Apr 1;32: 42-6.

Rose MT, Patti AF, Little KR, Brown AL, Jackson WR, Cavagnaro TR. A meta-analysis and review of plant-growth response to humic substances: practical implications for agriculture. Advances in agronomy. 2014; Jan 1;124:37-89.

Rouphael Y, Colla G. Biostimulants in agriculture. Frontiers in plant science. 2020; Feb 4;11:40.

Schiavon M, Pizzeghello D, Muscolo A, Vaccaro S, Francioso O, Nardi S. High molecular size humic substances enhance phenylpropanoid metabolism in maize (Zea mays L.). Journal of chemical ecology. 2010; Jun;36:662-9.

Shortell SM, Wu FM, Lewis VA, Colla CH, Fisher ES. A taxonomy of accountable care organizations for policy and practice. Health services research. 2014; Dec;49(6):1883-99.

Simard SW, Beiler KJ, Bingham MA, Deslippe JR, Philip LJ, Teste FP. Mycorrhizal networks: mechanisms, ecology and modelling. Fungal Biology Reviews. 2012; Apr 1;26(1):39-60.

Vranova V, Rejsek K, Skene KR, Formanek P. Non-protein amino acids: plant, soil and ecosystem interactions. Plant and Soil. 2011; May; 342:31-48.

Wally, O.S.D., Critchley, A.T., Hiltz, D., Craigie, J.S., Han, X., Zaharia, L.I., Abrams, S.R., Prithiviraj, B.,Regulation of phytohormone biosynthesis and accumulation in Arabidopsis following treatment with commercial extract from the marine macroalga Ascophyllum nodosum. J. Plant Growth Regul.2013; 32, 324–339.

Zhang and Schmidt, 1997.Biostimulants definition -wikipedia