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Review

Plant biostimulants: Definition, concept, main categories and regulation

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ABSTRACT

A plant biostimulant is 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. By extension, plant biostimulants also designate commercial products containing mixtures of such substances and/or microorganisms. The definition proposed by this article is supported by arguments related to the scientific knowledge about the nature, modes of action and types of effects of biostimulants on crop and horticultural plants. Furthermore, the proposed definition aims at contributing to the acceptance of biostimulants by future regulations, especially in the EU, drawing the lines between biostimulants and fertilisers, pesticides or biocontrol agents. Many biostimulants improve nutrition and they do so regardless of their nutrients contents. Biofertilisers, which we propose as a subcategory of biostimulants, increase nutrient use efficiency and open new routes of nutrients acquisition by plants. In this sense, microbial biostimulants include mycorrhizal and non-mycorrhizal fungi, bacterial endosymbionts (like *Rhizobium*) and Plant Growth-Promoting Rhizobacteria. Thus, microorganisms applied to plants can have a dual function of biocontrol agent and of biostimulant, and the claimed agricultural effect will be instrumental in their regulatory categorization. The present review gives an overview of the definition and concept of plant biostimulants, as well as the main categories. This paper will also briefly describe the legal and regulatory status of biostimulants, with a focus on the EU and the US, and outlines the drivers, opportunities and challenges of their market development.

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1. Introduction

The word biostimulant was apparently coined by horticulture specialists for describing substances promoting plant growth without being nutrients, soil improvers, or pesticides. Tracing back the first definition of the word biostimulants identifies a web journal dedicated to turf maintenance professionals, called *Ground Maintenance* (http://grounds-mag.com). In this web journal in 1997, Zhang and Schmidt from the Department of Crop and Soil Environmental Sciences of the Virginia Polytechnic Institute and State University defined biostimulants as *'materials that, in minute quantities, promote plant growth '*. By using the words *'minute quantities'* for describing biostimulants, the authors aimed at distinguishing biostimulants from nutrients and soil amendments, which also promote plant growth but are applied in larger quantities. The biostimulants mentioned by this web article are humic acids and seaweed extracts. Later peer-reviewed papers by the same authors on the same or similar research did not necessarily use the term biostimulant. For instance a paper describing the use of humic acis and seaweed extracts for increasing drought tolerance of turfgrass did not use the term biostimulant at all (Zhang and Schmidt, 2000). The paper focused on the hormone-like activities of these compounds and the term 'hormone-containing products' was used instead of biostimulants. This choice could also be explained by the regulation in the United States, where the Environmental Protection Agency ('EPA') exempts 'vitamin-hormone horticulture products' from registration under certain conditions. Zhang and Schmidt explained the biostimulation action by hormonal effects and, secondly, by protection against abiotic stress by antioxidants. The term 'metabolic enhancers' was also used in later papers (Zhang et al., 2003).

In the scientific literature, the word biostimulant was first defined by Kauffman et al. (2007) in a peer-reviewed paper, with modifications: 'biostimulants are materials, other than fertilisers, that promote plant growth when applied in low quantities.' Worth mentioning is the addition of the words 'other than fertilisers', which is in line with the description of Zhang and Schmidt, but which was not explicitely included in their original definition. Kauffman et al. (2007) attempt to summarize what biostimulants are, by introducing a classification: 'Biostimulants are available in a variety of formulations and with varying ingredients but are generally classified into three major groups on the basis of their source and content. These groups include humic substances (HS), hormone containing products (HCP), and amino acid containing products (AACP). HCPs, such as seaweed extracts, contain identifiable amounts of active plant growth substances such as auxins, cytokinins, or their derivatives'.

The word biostimulant was increasingly used by the scientific literature over the following years, expanding the range of substances and of modes of actions (Calvo et al., 2014; du Jardin, 2012; Halpern et al., 2015). In fact, 'biostimulant' appears as a versatile descriptor of any substance beneficial to plants without being nutrients, pesticides, or soil improvers. To some extent, biostimulants are first defined by what they are *not*, by drawing a borderline between biostimulants and other widely used categories of substances applied to plants and crops: fertilisers and pesticides. In a second stage, it turned out that the positive actions ascribed to the chemical biostimulants (of natural or synthetic origin) – growth promotion, modulation of development and of quality traits, increased tolerance to environmental stress – can also be delivered by bacteria and fungi. As an example, PGPRs or 'plant growth-promoting rhizobacteria' are defined by beneficial effects on the plants, without being nutrients, pesticides or soil improvers. Like chemical substances, their nature (i.e. their taxonomic status in this case) can be very diverse and the PGPR category is defined on the basis of its agricultural/horticultural outputs. 'Biofertilisers' and 'biocontrol agents' are also used for describing PGPRs, refering to the expected agricultural /horticultural outputs. The relationships between these concepts and terms will be discussed later in this paper.

Industry is a key player in the definition and promotion of the concept of biostimulants, including microorganisms. Companies in the sector have created associations, like the 'European Biostimulants Industry Council' (EBIC) in Europe and the 'Biostimulant Coalition' in the USA, dialoguing with other stakeholders, regulators and scientists. The corporate sector has also supported the organisation of international symposiums. The *'First World Congress on the use of Biostimulants in agriculture'* took place in Strasbourg, in November 2012 and may be regarded as a milestone in the acceptance of biostimulants into the academic area.

The purpose of this article is to contribute to a better understanding of the concept of plant biostimulant on the basis of the theoretical and practical knowledge of the main categories of biostimulant products used in agriculture and horticulture. With this aim, the main categories will be briefly described. Their modes of action will be summarized, providing the basis of any definition.

2. Main categories of plant biostimulants

Despite recent efforts to clarify the regulatory status of biostimulants, there is no legal or regulatory definition of plant biostimulants anywhere in the world, including in the European Union and in the United States. This situation precludes a detailed listing and categorization of the substances and microorganisms covered by the concept. Despite this, some major categories are widely recognized by scientists, regulators and stakeholders (Calvo et al., 2014; du Jardin, 2012 ; Halpern et al., 2015), covering both substances and microorganisms. Microorganisms include beneficial bacteria, mainly PGPRs, and beneficial fungi. They can be free-living, rhizospheric or endosymbiotic. These categories are briefly introduced in the next section and will be further described by the accompanying papers of this special issue on plant biostimulants in horticulture.

2.1. Humic and fulvic acids

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 sustances – 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 (Rose et al., 2014) concluded on an overall dry weight increase of 22 ± 4% for shoots and of by 21 ± 6% for roots.

The variability in effects of HS are due to the source of the HS, the environmental conditions, the receiving plant and the dose and manner of HS application (Rose et al., 2014). Regarding the sources of HS (du Jardin, 2012), they are extracted from naturally humified organic matter (*e.g.* from peat or volcanic soils), from composts and vermicomposts, or from mineral deposits (leonardite, an oxidation form of lignite). Furthermore, agricultural by-products, instead of being decomposed in a soil or by composting, are amenable to controlled breakdown and oxidation by chemical processes, leading to 'humic-like substances' which are proposed as substitute for natural HS (Eyheraguibel et al., 2008).

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 biostimulant 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 biostimulation 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 (Olivares et al., 2015; Schiavon et al., 2010). .

2.2. *Protein hydrolysates and other N-containing compounds*

Amino-acids and peptides mixtures are obtained by chemical and enzymatic protein hydrolysis from agroindustrial 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 biostimulants of plant growth (Calvo et al., 2014; du Jardin, 2012, Halpern et al., 2015). 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 (Colla et al., 2014). 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 nitrogeous compounds, including glycine betaine and proline, which contributes to the mitigation of environmental stress.

Indirect effects on plant nutrition and growth are also important in the agricultural practice when protein hydrolysates are applied to plants and soils. Protein hydrolysates are known to increase microbial biomass and activity, soil respiration and, overall, soil fertility. Chelating and complexing activities of specific amino acids and peptides are deemed to contribute to nutrients availability and acquisition by roots.

Several commercial products obtained from protein hydrolysates of plant and animal origins have been placed on the market. Variable, but in many cases significant improvements in yield and quality traits have been reported in agricultural and horticultural crops (Calvo et al., 2014). The safety of hydrolyzed proteins of animal origin was recently assessed and no genotoxicity, ecotoxicity or phytotoxicty was reported on the basis of bioassays using yeasts and plants as test organisms (Corte et al., 2014). Nevertheless, there is a growing safety concern of using protein hydrolysates derived from animal by-products in the food chain. The EU banned the application of such animal protein hydrolysates on the edible parts of organic crops, through the Commission Implementing Regulation (EU) no 354/2014 with regard to organic production, labelling and control.

2.3. *Seaweed extracts and botanicals*

The use of fresh seaweeds as source of organic matter and as fertiliser is ancient in agriculture, but biostimulant 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 carrageenans 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 carrageenans originate from red seaweeds, which correspond to a distinct phylogenetic line. Product names of more thant 20 seaweed products used as plant growth biostimulant have been listed by Khan et al. (2009).

Seaweeds act on soils and on plants (Craigie et al., 2008; Craigie, 2011; Khan et al., 2009). They can be applied on 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 biostimulation activity on crop plants. Although cytokinins, 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., 2013a,b). Molecular genetics, *i.e.* hormone mutants in *Arabidopsis* and transcript analysis by RT-qPCR, were used to reach this conclusion.

Anti-stress effects are also reported and both protective compounds within the seaweed extracts, like antioxidants, and regulators of endogenous stress-responsive genes could be involved (Calvo et al., 2014).

‘Botanicals’ describe substances extracted from plants which are used in pharmaceutical and cosmetic products, as food ingredients, and also in plant protection products (Seiber et al., 2014). Compared with seaweeds, much less is known regarding their biostimulant activities, the attention being focused on their pesticidal properties so far. However, there seems to be opportunities to use them as biostimulants as well (Ertani et al., 2013; Ziosi et al., 2012). Furthermore, plant interactions in ecosystems are known to be mediated by plant active compounds, refered to as allelochemicals, which are receiving increasing attention in the context of sustainable crop management. Although crop rotations, intercropping, cover crops and mulching are used in the first instance to exploit allelochemical interactions between plants (named allelopathy), futher attention should be paid to these chemical interactions for the development of new biostimulants.

2.4. 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 defense gene activation, in a similar way as plant defense elicitors (El Hadrami et al., 2010; Hadwiger, 2013; Katiyar et al., 2015; Yin et al., 2010). 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 on quality traits related to primary and secondary metabolisms. Stomatal closure induced by chitosan via an ABA-dependent mechanism (Iriti et al., 2009) participates to the environmental stress protection confered by this biostimulant.

Several poly- and oligomers of biological origin or (hemi-) synthetic variants are increasingly used in agriculture as elicitors of plant defense, including seaweed polysaccharides which we have already mentioned. 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 biostimulation (*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).

2.5. 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 (Pilon-Smits et al., 2009). 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 lik amorphous silica ($SiO_2.nH_2O$) in graminaceaous 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 biostimulants, like extracts of seaweeds, of crop residues or animal wastes, involves the physiological functions of the contained beneficial elements.

Many effects of beneficial elements are reported by the scientific litterature, 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 (Pilon-Smits et al., 2009).

Inorganic salts of beneficial and essential elements – chlorides, phosphates, phosphites, silicates and carbonates – have been used as fungicides (Deliopoulos et al., 2010). Although the modes of action are not yet fully established, these inorganic compounds influence osmotic, pH and redox homeostasis, hormone signalling and enzymes involved in stress response (*e.g.* peroxidases). Their function as biostimulant of plant growth, acting on nutrition efficiency and abiotic stress tolerance, hence distinct from their fungicidal action and from their fertiliser function as sources of nutrients, deserves more attention.

2.6. Beneficial fungi

Fungi interact with plant roots in different ways, from mutualistic symbioses (*i.e.* when both organisms live in direct contact with each other and establish mutually beneficial relationships) to parasitim (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; Johnson and Graham, 2013). Mycorrhizal fungi are a heterogeneous group of taxa which establish symbioses with over 90 % of all plant species. 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; Siddiqui et al., 2008; van der Heijden et al., 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; Simard et al., 2012). As a further area of research, AMF form tripartite associations with plants and rhizobacteria which are relevant in practical field situations (Siddiqui et al., 2008). In order to reap the benefits of the mycorrhizal associations, crop management practices and plant cultivars should be adapted to the interaction with microorganisms (Gianinazzi et al., 2010; Hamel and Plenchette, 2007; Plenchette et al., 2005; Sheng et al., 2011). Metagenomics are an interesting tool to monitor and study microbial associations in the rhizosphere. Inoculation of plant propagules and soils complements these approches (Candido et al., 2013, 2015; Colla et al., 2015; Sarkar et al., 2015; Sensoy et al., 2007; Sorensen et al., 2008).

Fungal-based products applied to plants to promote nutrition efficiency, tolerance to stress, crop yield and product quality should fall under the concept of biostimulants. 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 biopesticidal (mycoparasitic) and biocontrol (inducer of disease resistance) capacities, and have been exploited as sources of enzymes by biotechnological industries (Mukherjee et al., 2012; Nicolás et al., 2014). 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; Shoresh et al., 2010). On the basis of these effects, these fungal endophytes may be regarded as biostimulants, though their agricultural uses are currently supported by claims as biopesticides.

2.7. *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 my 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 biostimulants, 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 biofertilisers, *i.e.* microbial inoculants facilitating nutrients acquisition by plants (see glossary in Box 1). The biology and agricultural uses of the *Rhizobium*-based symbioses have been extensively reviewed by the scientific literature and in textbooks. 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; Bhattacharyya and Jha, 2012; Gaiero et al., 2013; Philippot et al., 2013; Vacheron et al., 2013). 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 give rise to inconsistent results in practice (Arora et al., 2011; Brahmaprakash and Sahu, 2012). Despite this, the world market of bacterial biostimulants is growing and PGPR inoculants are now regarded as some kind of plant 'probiotics', *i.e.* efficient contributors to plant nutrition and immunity (Berendsen et al., 2012).

Box 1: Glossary of 'biosolutions' contributing to sustainable plant productions

Biostimulant: A plant biostimulant is 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. By extension, plant biostimulants also designate commercial products containing mixtures of such substances and/or microorganisms.

Biofertiliser: A biofertiliser is any bacterial or fungal inoculant applied to plants with the aim to increase the availability of nutrients and their utilization by plants, regardless of the nutrient content of the inoculant itself. Biofertilisers may also be defined as microbial biostimulants improving plant nutrition efficiency.

Biocontrol: The control of one organism by another. Biocontrol agents used in plant productions are living organisms protecting plants against their enemies, i.e. reducing the population of pests or diseases to acceptable levels. Modes of action may include competition, antibiosis, parasitism and also Induced Systemic Resistance which is mediated by the plant.

3. Common features of biostimulants

A common designation of biostimulants is only justified if the described substances and microorganisms share some important characteristics regarding their natures, functions and/or uses. Such characteristics would then be the ground for any definition.

From the bibliographic review, the following conclusions may be drawn:

1. *The nature of biostimulants is diverse*. Substances and microorganisms are involved. Substances can be single compounds (*e.g.* glycine betaine) or groups of compounds of single natural origin of which the composition and bioactive components are not fully characterized (*e.g.* seaweed extracts); the substances commented by this review are naturally produced organic compounds, or inorganic molecules, but synthetic compounds should not be excluded, especially if certain plant growth regulators are included within biostimulants (for example, nitro-phenolates are described and commercialized as 'biostimulants' but are synthetic phenolic compounds registered as plant production products according to the EU Law, see Przybysz et al., 2014). Microbial inoculants may contain single strains (*e.g.* of *Bacillus subtilis*) or mixtures of microorganisms showing additive or synergistic effects (*e.g.* several products on the market). Depending on the peer-reviewed and 'grey' scientific literature and on the documentation provided by companies, biostimulants may refer to the bioactive ingredients or to the commercialized products combining them and often added to fertilisers or crop protection products. Any regulatory definition will have to clarify whether ingredients or final products (or both) are actually covered.
2. *The physiological functions are diverse*. By physiological function, we mean any action on plant processes (Table 1). Examples of physiological functions are the protection of photosynthetic machinery against photodamage, or the initiation of lateral roots. Functions are supported by cellular mechanisms, like reactive oxgen scavenging by antioxidants or increased synthesis of auxin transporters, to carry on with the two previous examples. Phys-

Table 1
Effects of biostimulants on crop productions, from their cellular targets in plants to whole-plant physiological functions, to agricultural/horticultural functions, and ultimately to expected economic and environmental benefits (Dobbelaere et al., 1999; Huang et al., 2010; Shabala et al., 2012).

	Humic acids	**Seaweed extracts**	**Protein hydrolysate**	**Glycine betaine**	**Plant Growth-promoting Rhizobacteria**
Cellular mechanism (*i.e.* interaction with cellular components and processes) ↓	Activate plasma membrane proton-pumping ATPases, promote cell wall loosening and cell elongation in maize roots (*Zea mays*) (Jindo et al., 2012)	*Ascophyllum nodosum* extracts stimulate expression of genes encoding transporters of micronutrients (*e.g.* Cu, Fe, Zn) in oilseed rape (*Brassica napus*) (Billard et al., 2014)	Enzymatic hydrolysate from alfalfa (*Medicaco sativa*) stimulates phenylalanine ammonia-lyase (PAL) enzyme and gene expression, and production of flavonoids under salt stress (Ertani et al., 2013)	Protects photosystem II against salt-induced photodamage in quinoa (Shabala et al., 2012), likely via activation of scavengers of reactive oxygen (Chen & Murata , 2011)	*Azospirillum brasilense* releases auxins and activates auxin-signalling pathways involved in root morphogenesis in winter wheat (*Triticum aestivum*) (Dobbelaere et al., 1999)
Physiological function (*i.e.* action on whole-plant processes) ↓	Increased linear growth of roots, root biomass	Increased tissue concentrations and root to shoot transport of micronutrients	Protection by flavonoids against UV and oxidative damage (Huang et al., 2010)	Maintenance of leaf photosynthetic activity under salt stress	Increased lateral root density and surface of root hairs
Agricultural/horticultural function (*i.e.* output traits relevant for crop performance) ↓	Increased root foraging capacity, enhanced nutrient use efficiency	Improved mineral composition of plant tissues	Increased crop tolerance to abiotic (*e.g.* salt) stress	Increased crop tolerance to abiotic (*e.g.* high salinity) stress	Increased root foraging capacity, enhanced nutrient use efficiency
Economic and environmental benefits (*i.e.* changes in yield, products quality, ecosystem services)	Higher crop yield, savings of fertilisers and reduced losses to the environment	Enhanced nutritional value, 'biofortification' of plant tissues (increased contents in S, Fe, Zn, Mg, Cu)	Higher crop yield under stress conditions (*e.g.* high salinity)	Higher crop yield under stress conditions (*e.g.* high salinity)	Higher crop yield, savings of fertilisers and reduced losses to the environment

iological functions and the underlying cellular mechanisms may be refered to as 'modes of actions' of the biostimulants, collectively. Finally, these modes of actions explain the agricultural functions of biostimulants, *e.g.* increased tolerance to abiotic stress (causing oxidative stress), or increased N use efficiency (which depends of the foraging capacity of roots, hence on lateral root density). Agricultural functions may finally translate into economic and environmental benefits: higher crop yield, savings of fertilisers, increased quality and profitability of crop products, enhanced ecosystem services, etc.

3. The scientifically demonstrated effects of all biostimulants converge to *at least one or several of the following agricultural functions*: they enhance nutrition efficiency, abiotic stress tolerance and/or crop quality traits. Quality traits may refer to nutritional value, grain protein content, shelf life, etc. These converging actions should be the basis of any definition of biostimulants. Stimulation of pathogen response by elicitors and plant gene regulators is achieved by many of the described biostimulants as well (chitosan, laminarin, some PGPRs, etc.). However, there is a growing consensus among regulators and stakeholders to keep biostimulation and biocontrol separate from a regulatory point of view. Biotic stress is taken out of the scope of the definition, accordingly.
4. *Definition of economic and environmental benefits depends on agricultural and environmental policies, both in terms of objectives and assessment endpoints*. Although incentives for developing biostimulants are linked to these aspects, they should not be the ground for a science-based definition of biostimulants.

In conclusion any definition of biostimulants should focus on the agricultural functions of biostimulants, not on the nature of their constituents nor on their modes of actions, as they have been defined above.

4. Defining plant biostimulants : aiming at a consensus

In line with the above considerations, the following definition is proposed (Box 1):

« **A plant biostimulant is 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.** » This definition could be completed by : « By extension, plant biostimulants also designate commercial products containing mixtures of such substances and/or microorganisms.»

A couple of remarks :

1. The nature of the biostimulant is not restrictive : it can be a substance or a microorganism. A substance may be either a single chemical compound or a group of compounds having a well established biological origin, *e.g.* plant extracts, but not necessarily a fully characterized composition. In this sense, it fits with the meaning of the word « substance » in existing reg-

ulations. This includes the european REACH regulation (EC No 1907/2006) concerning the Registration, Evaluation, Authorisation and Restriction of Chemicals, which recognizes a category of substances of variable composition : *'UVCB substances (substances of unknown or variable composition, complex reaction products or biological materials) may be registered as a single substance under this Regulation, despite their variable composition, provided that the hazardous properties do not differ significantly and warrant the same classification'*. Another example of complex substances potentially comprising many chemical constituents are plant extracts refered to as 'botanical active substances ' and as ' basic substances ' and approved under regulation (EC) No 1107/2009 on plant protection in the EU. The European Commission 'guidance document on the botanical active substances used in plant protection product ' (http://ec.europa.eu/food/plant/pesticides/guidance_documents/docs/guidance_document_botanicals_rev_8_en.pdf) defines: *'A 'botanical active substance' consists of one or more components found in plants and obtained by subjecting plants or parts of plants of the same species to a process such as pressing, milling, crushing, distillation and/or extractions'*. Clearly, the multicomponent nature of substances of plant origin is acknowledged here, as it is in international forums of the OECD on biopesticides (http://www.oecd.org/env/ehs/pesticides-biocides/env-jm-mono-2012-36-core%20report.pdf). The word substance in the definition of biostimulants should be understood in a similar way. Microorganisms should be identified at the level of the strain, considering that many biological activities are indeed strain-specific. When mixtures (*i.e.* intentional blends) of microorganisms are used, the resulting products would be refered to as biostimulants, following our proposal to extend the definition to commercial preparations.

2. The agricultural functions form the core of the definition. Biostimulants are defined by intended agricultural outputs. 'Nutrition efficiency' may cover nutrient mobilization and uptake from the soil, root development, transport, storage and assimilation (*i.e.* conversion of inorganic to organic forms) of nutrients in the plant. 'Abiotic stress' refers to any physical or chemical stressor of non biological origin (drought, salinity, cold, etc.). 'Quality traits' may be diverse and range from nutritional value to shelf life or flower pigmentation. Any of these effects should be distinct from those resulting from the nutrient content of the biostimulant. Biostimulants are not fertilisers in the sense they do not contain nutrients intended to be delivered to the plant. However, they may facilitate nutrient acquisition, *e.g.* by mobilizing elements in the rhizosphere or by developing new routes of nutrient acquisition, like fixation of atmospheric N by the recruitment of bacterial endosymbionts.
3. The proposed definition is in line with the few existing definitions under discussion between regulators and the industry. The association EBIC proposes the following: *'Plant biostimulants contain substance(s) and/or micro-organisms whose function when applied to plants or the rhizosphere is to stimulate natural processes to enhance/benefit nutrient uptake, nutrient efficiency, tolerance to abiotic stress, and crop quality '*. This is consistent with the proposal of this article. However, biostimulants applied to the rhizosphere can be covered by the wording 'applied to plants' (fertilisers and pesticides can also be described as being applied to plants even if they are sprayed on the parcel, including plants and soils, including the rhizosphere). By saying 'applied to plants' in our definition, the intention is not to be restrictive, but on the contrary to cover all modes of application targeting the plant at the end. Furthermore, a biostimulant is definedas being primarily the substance and/or the microorganism exerting some effect, not what contains substances and microorganisms. EBIC seems to refer in the first instance to the commercialized product containing active ingredients. a biostimulant is defined for designating the active substance or microorganism in the first place, and secondly any commercial preparation containing them.

In the US, the Biostimulant Coalition, a group of interested parties equivalent to the EBIC, has attempted to coalesce around a definition and to reach an agreement with the American Association of Plant Food Control Officials (AAPFCO), which is instrumental in harmonizing fertilizer and soil amendment laws between States. Unfortunately, no agreement on a definition of biostimulants could be reached. However, a breakthrough took place in February 2014 when AAPFCO agreed to expand the definition of the existing category of 'beneficial substances' in order to include biostimulants. Beneficial substances are defined as "*any substance or compound other than primary, secondary, and micro plant nutrients that can be demonstrated by scientific research to be beneficial to one or more species of plants, when applied exogenously.*" (AAPFCO, 2012) As such, many important biostimulants (*e.g.* all microbial biostimulants) are excluded from the definition. The current approach aims at including biostimulants as subcategories of beneficial substances, each having its own specification and definition. The initial subcategories proposed by the Biostimulant Coalition are: antioxidants, amino acids materials, biomolecule/biomolecular, enzymatic extracts, fulvic acid materials, humic acid materials, microbial inoculants, microbial soil amendments, mycorrhizal fungi, PGPRs, phytohormones, seaweed extract materials.

5. Regulation of plant biostimulants

The regulatory situation of biostimulants is very complex today, in the absence of any specific and harmonized framework in either the EU or the USA. One of the main reasons for this situation is the lack of formal definition and acceptance of the concept by regulatory bodies. In Europe today, biostimulants are placed on the market by following either of two routes : one is the national regulations on fertilisers, the other one is the european pesticides law, which combines both supranational and national provisions for introducing plant protection products on the market. In Europe, the current situation is that the EC regulation No 1107/2009 on plant protection products ('PPPs') is applicable to all categories of biostimulants, considering the very broad definition of PPPs. Indeed, Article 2 of this regulation reads : '*This Regulation shall apply to products, in the form in which they are supplied to the user, consisting of or containing active substances, safeners or synergists, and intended for one of the following uses:*

(a) *(. . .)*

(b) *influencing the life processes of plants, such as substances influencing their growth, other than as a nutrient.*'

As any biostimulant is intended to influence the life processes of plants by other ways than as a nutrient, it may be regarded as a « plant protection product » from a strict regulatory viewpoint. Synthetic and natural substances (including botanicals and basic substances as mentioned before), and microorganisms, are all covered by this regulation. All plant growth regulators and herbicide safeners have been registered under this PPP regulation until now and these are substances that interact with the physiology of the plant, even though they do not protect the plant against pests or diseases.

Due to the lengthy and costly procedures to place a PPP on the european market, taking into consideration that many companies developing biostimulants are SMEs and that improved plant nutrition and growth are the main scope of biostimulants, an alternative route has been chosen, namely the 'fertilisers route' in which case national legislation is applied. Why not the european law on EC fertilisers (regulation (EC) No 2003/2003) ? Because the definition of fertilisers laid down by this regulation is very restrictive and cannot include biostimulants. Indeed, Article 2 reads:

'For the purposes of this Regulation the following definitions shall apply:

(a) *'Fertiliser' means material, the main function of which is to provide nutrients for plants.*
(b) *'Primary nutrient' means the elements nitrogen, phosphorus and potassium only.*
(c) *'Secondary nutrient' means the elements calcium, magnesium, sodium and sulphur.*
(d) *'Micro-nutrients' means the elements boron, cobalt, copper, iron, manganese, molybdenum and zinc essential for plant growth in quantities that are small compared with those of primary and secondary nutrients.'*

Any fertiliser must provide nutrient as its main function. This is clearly not the case of biostimulants, which by definition promote plant growth by other means than by providing nutrients. Annex I of the (EC) No 2003/2003 regulation on EC fertilisers lists types of fertilisers, which are all inorganic materials providing macro- and micronutrients, but also chelating and complexing agents intended to optimize the delivery of micronutrients to plants, allowing chelated and complexed micronutrients to be placed on the market by the way of this regulation. It was later considered that other compounds used as fertilisers additives, *i.e.* nitrification and urease inhibitors, should also be granted market access via this regulation. This led to a breakthrough in the european fertiliser regulation, which was amended by the (EC) No 1107/2008 regulation in order to introduce materials which are not providers of nutrients (fertilisers *sensu stricto*) but additives of fertilisers enhancing fertilisers performance. Many biostimulants may be considered as enhancers of fertilisers performance and this regulatory advance seemed to pave the way to the inclusion of biostimulants into the EU fertilisers law. However, this option is not realistic as amending regulations is a laborious procedure which cannot be followed for all biostimulants. When the national fertilisers laws are used for introducing biostimulants on the european market (mainly those enhancing nutrition and growth, *e.g.* humic acids, seaweed extracts and protein hydrolysates), marked differences exist between member states in terms of data requirements for efficacy, toxicity and ecotoxicity assessment (Traon et al., 2014; La Torre et al., 2015). To complete this overview, it is worth to mention that legal provisions exist within the EU to promote « mutual recognition » between member states (Regulation (EC) No 764/2008), *i.e.* 'fast tracks' exist for the placing on the market of members states when an authorisation has been granted in one of them. However, based on interviews with representatives of stakeholders and competent authorities, It is realistic to think that this system is not efficient enough and is not expected to develop in the future.

Taking into consideration the need for harmonization of legislation on biostimulants, but also of other categories of fertilising materials and additives, – *i.e.* nutrients performance enhancers, organic and organo-mineral fertilisers, soil improvers, growing media, liming materials – the European Commission and its Fertilisers Working Group representing competent authoritites of member states and stakeholders has initiated an ambitious reform of its fertilisers regulation (see public reports at http://ec.europa.eu/transparency/regexpert/index.cfm?do=groupDetail.groupDetail&groupID=1320).

The situation in the USA is to some extent similar to the european situation : no approved definition of biostimulants, no harmonization between the 50 states, use of fertilisers laws for the placing on the market of certain biostimulants at the state level, and work in progress between stakeholders, representatives of regulatory bodies and federal agencies to improve the legal certainty surrounding biostimulants. The role of the American Association of Plant Food Control Officials has already been underlined, especially regarding the definitions and formal recognition of categories of fertilisers (AAPFCO, 2012). The future will indicate how the federal agencies EPA and USDA will regulate biostimulant products. A plausible scenario today seems that some of the biostimulants could fall under EPA jurisdiction, while the others would be registered as fertilisers or soil amendments at the state level (David Beaudreau, Biostimulant Coalition, personal communication). Furthermore, USDA, via its Natural Resources Conservation Service (NRCS) Agency, could acknowledge the capacity of certain biostimulants to reduce nutrient run off by including biostimulant products in a list of soil health-promoting practices.

6. Developing the market : opportunities and challenges

Due to the lack of legal acceptance of the concept of biostimulants, maket data are scarce and of limited reliability. The regulatory status of biostimulants is indeed diverse, depending on whether or not they are registered under the REACH regulation, as fertilising materials under national laws, as pesticides under european legislations, authorized or not in organic productions, etc. Biostimulants are spread over many regulations, without being named as such, and this situation constraints the establishment of registers of products and of reliable statistics of their uses. Still, the association EBIC has issued economic overviews of the biostimulants sector in Europe, based on surveys of its members (EBIC, 2013). Although the data are qualitative rather than statistical, as acknowledged by EBIC, they indicate a steadily growing market (of about 10% or more per year), whatever indicator is used (sales, treated hectares, number of users). The main crops on which biostimulants are applied today in Europe are indicated in Table 2. Another market analysis report was issued recently, which seems to confirm the outlines by EBIC (Marketsandmarkets.com, 2014, see also at www.agra-net.com).

It is problably more relevant to identify the drivers of this growing interest. The main drivers are related to general agricultural and environmental policies, but other driving factors are more specific to the biostimulants sector. Regarding the first aspect, there is an increasing awareness of the need to promote sustainable agriculture worldwide, combining high productivity and high resource use efficiency (Garnett et al., 2013; SCAR, 2011; The Government Office for Science, 2011; Tilman et al., 2002).

Productive and resource efficient agrosystems should face future needs for food and non food materials, but they should also deliver ecosystem services wich contribute the preservation of soils, water and air. Sustainable intensification of crop productions calls for the recruitment of ecological functionalities in case of protection and nutrition of plants (Box 1). Biocontrol agents (*e.g.* predator or parasitoid insects, antagonistic bacteria producing toxins and antibiotics, etc.) and biofertilisers (*e.g.* root growth-promoting, N-fixing PGPRs) benefit plants plants via ecological interactions in the cultivated ecosystem. Organic farming and agroecology promote the use of such biological solutions and of materials of biological origin. In Europe, several biocontrol agents and biostimulants are included in registers of materials authorized in organic production (Regulation (EC) No 889/2008). Inclusion in these registers is not equivalent to a marketing authorization, which depends on separate regulations on fertilisers and plant protection products.

Regarding the factors related to the biostimulants sector, the EBIC surveys and market analyses point to the spread of biostimulants to new geographical areas and new crop productions, from the pioneer countries and application sectors (*e.g.* from horticultural to agricultural crops, from organic to conventional productions).

Table 2
Indicative list of some crops to which biostimulants are currently applied in Europe (adapted from EBIC, 2013, with permission).

Trees and vine crops	Agricultural crops	Vegetable and legume crops		Other horticultural crops
Citrus	Barley	Broccoli	Onions	Ornamentals
Pome fruits	Maize	Cabbage	Peppers	Nursery
Stone fruits	Rice	Carrots	Potato	Turf
Grapes (table)	Wheat	Cauliflower	Lettuce	
Grapes (wine)	Oilseed rape	Cucumber	Squash	
	Sugar beet	Eggplant	Strawberry	
		Garlic	Tomato	
		Melons	Watermelon	

Another driver of market development is the relatively high investment of companies in research and development (between 3% and 10% of the annual turnover in the surveyed members of EBIC), which contributes to an expanding list of biostimulants and of industrial processes for their production and formulation.

Challenges for the development of biostimulants are of scientific, technical and regulatory nature.

The main scientific challenge is the complexity of the physiological effects of biostimulants. In general terms, the primary effects of biostimulants are to induce physiological responses in the plant. Many of these responses bear on primary metabolism, growth and development. These processes are subject to tight homeostatic regulations which originate from millions of years of biological evolution and explain why plants occupy specific ecological niches and display characteristic phenotypic responses to fluctuating environments. Acting on such biological processes is challenging and attention should be paid to the many cross talks between processes and pathways in plant organisms in their response to their environment. Furthermore, the use of biostimulants can only be successful if the tripartite interactions between the biostimulant, the plant and the environment can be properly addressed. As an example, phosphorus mobilization by the help of phosphatase-releasing PGPRs may contribute to plant growth and crop yield if soil inorganic phosphate is indeed limiting and if the plant contributes to the maintenance and activity of the PGPR inoculant in the rhizosphere, *e.g.* via its exudates.

Technical challenges include the formulation and blending of biostimulants with other fertilising materials and/or plant protection products. Many biostimulants aim at improving nutrient use efficiency and combinations between fertilisers and biostimulants will need to be optimized. Formulation of biofertilisers is specially complex, and positive interactions between microbiological components of the biostimulants mixtures on one hand, and between the biostimulant inoculant and the resident rhizo-/endospheric microbiota on the other hand, have to be searched for. Technical difficulties also arise for the monitoring of crops and for deciding on whether, when and how biostimulants should be applied. The situation is more complex than with any plant protection products, for which the incidence of pests and diseases is relativey easy to detect and quantify, and for which epidemiological models are available to optimize pesticides applications. We are far from such a situation with abiotic stressors, which often interact in the field, are difficult to assess and are usually quantified *ex post* by yield penalties. Nutrient use efficiency is also difficult to evaluate and decision to apply a biostimulant that would target this trait is hardly justified by measurable plant characteristics in the field. Biostimulants look like a « nice-to-have » technology instead of a « need-to-have » technology (as compared with pesticides) and consideration of both immediate and delayed benefits to the farmers, including resource savings and ecosystem services, should be promoted in the long term.

Regulatory challenges are related to the categorization and premarket assessment of biostimulants, and to intellectual property. It is so far unclear whether and where biostimulants will be regulated as such, *i.e.* recognized as a specific regulatory category by national or supranational laws. Current laws on fertilisers and pesticides allow for the safe placing on the market of biostimulants in most places of the world, and reasons for making them a distinct regulatory category should be made explicit. One such argument is the peculiarity of biostimulants regarding their mode of action – via the plant response- and of the intended effects – on nutrient use efficiency, abiotic stress tolerance, product quality – which may justify specific provisions for efficacy assessment. Providing guidance and ensuring regulatory certainty in the efficacy assessment, besides risk assessment, of biostimulants is important for developing the technology and the market, and this could be streamlined by supranational regulations. Another argument is market harmonization, as linking biostimulants to fertilisers has led so far to a great heterogeneity in the way they are placed on the market from one country to the other. Furthermore, developing a *sui generis* approach for biostimulants could help regulators optimize data bridging with existing regulations and registers, like those of the REACH regulation (EC No 1907/2006 concerning the registration, evaluation, authorisation and restriction of chemicals).

Biostimulants pose specific difficulties for the protection of intellectual property. Patentability and prevention of copies/reverse engineering of the biostimulant products are often difficult. Originality of the product and its status of invention, as demanded by patentability, is sometimes difficult to establish, and companies tend to patent the industrial processes used for the production of biostimulants. Data protection mechanisms coupled to the mandatory registration of biostimulants would strengthen protection of intellectual property. Data sharing mechanisms linked to the registration of biostimulant substances or microorganisms would support the development of the biostimulants market by promoting industrial exchanges and partnerships. The REACH regulation in Europe has paved the way for such development, but it covers chemicals and excludes microorganisms, which are important categories of biostimulants.

7. Concluding remarks – looking ahead

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. Bridging the gap between many laboratory data on single biostimulants and field data on mixtures (often combined with fertilisers) is equally challenging and important. As an example,

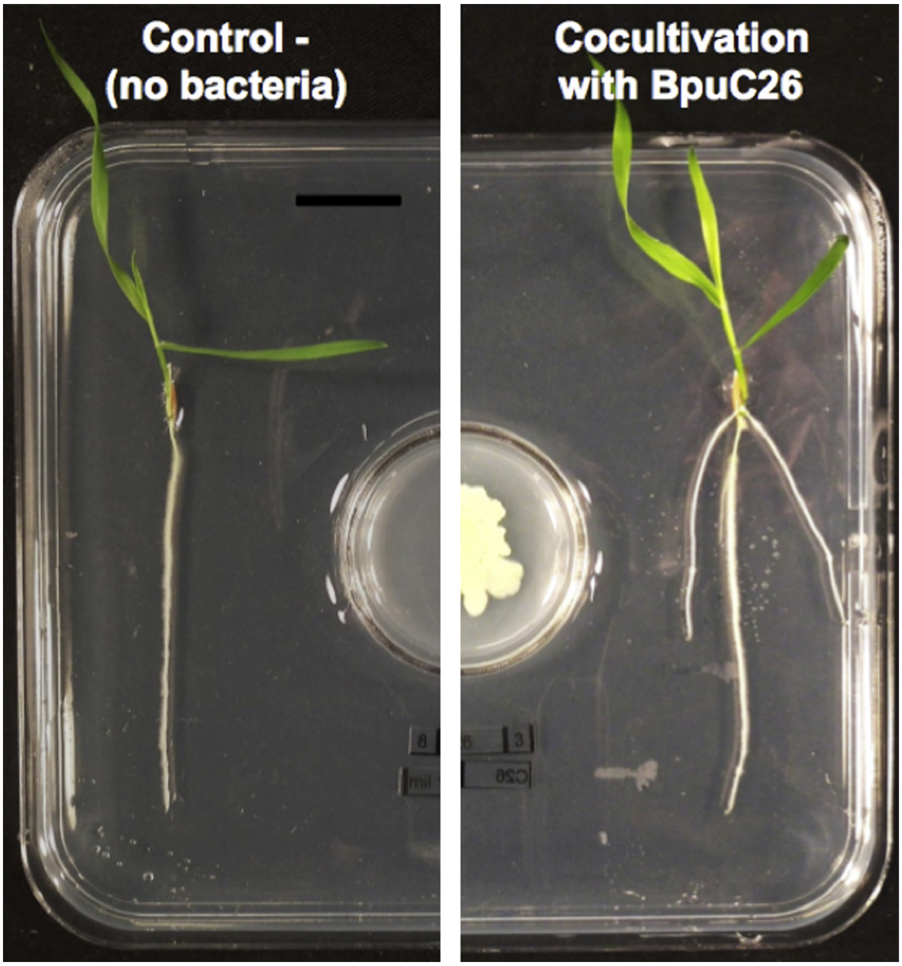

Fig. 1. Induction of lateral root formation on seedlings of the model grass *Brachypodium distachyon* (line Bd21) by volatile compounds emitted by the PGPR *Bacillus pumilus* C26, co-cultivated on a vertical plate within a shared atmosphere (Delaplace et al., 2015).

root growth promotion by soil bacteria can be consistently demonstrated in laboratory conditions (Fig. 1), but this says little about possible beneficial effects in practical field situations.

From the field to the laboratory: the development of biostimulants may follow a classical 'pharmacological' approach, where candidate active substances or microorganisms are screened in controlled conditions and a stepwise procedure is followed for selecting promising candidates, moving from the laboratory to more realistic conditions. This can be efficient but the stringent and stepwise selection of active substances or microorganisms results in high development costs which are hardly justified in market sectors creating limited added value, like in plant nutrition and agriculture. An alternative way would start from field observations and lead back to the laboratory for the systematisation of the scientific questions raised. To give an example, soil microbiologists and ecologists are pointing out the variability in the way individual plant cultivars interact with rhizospheric bacteria and modulate the composition of the bacterial populations, even over the growing season of an annual crop like maize (Aira et al., 2010; Philippot et al., 2013). Whether these genotype-dependent changes in the rhizospheric microbiome impact plant growth and health is an open question. Such observations can be a starting point for understanding the keys to successful interactions between PGPRs and plants. From a more practical viewpoint, novel commercial approaches are being developed which aim at amplifying local beneficial microbiota instead of inoculating standardized microbial products. This approach is motivated by the empirical fact that a limiting factor when using microbial biostimulants is the capacity of the inoculant to establish and maintain sufficient activity in the rhizosphere. A parallel can be made with the intestinal microbiota in human medecine : adding inoculants (*i.e.* 'probiotics') is one thing, but feeding beneficial bacteria with prebiotics seems even more important. Health benefits can be obtained by using prebiotics alone, which modulate the intestinal microbiome (Rastall and Gibson, 2015). This inspires new avenues to sustainable crop management, by developing new fertilising materials and by breeding plants with enhanced capacity to 'manage' their rhizospheric and endospheric microbiota.

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. Companies developing biostimulants will have to contribute to integrated solutions at the agrosystem, farm and landscape levels, of which biostimulants are only one element. 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. On this long way, public action is awaited to harmonise policies and regulations, and to build up a robust risk assessment framework which respects the principle of proportionality and avoids duplication of data requirements across regulations.

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