The Science of Plant Biostimulants – A bibliographic analysis

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Summary

Various substances and materials, when applied to plants or growing substrates, have a demonstrated capacity to modify the physiology of plants, promoting their growth and enhancing their stress response. At first glance, their action is distinct from that of nutrients and of plant pesticides and the term "biostimulants" has been coined to describe their function. At the request of the European Commission, the present study aims at analysing what biostimulants are, on the basis of the scientific literature, and to propose a scientifically sound definition. This definition should then be compared with the existing definitions and claims pertaining to the EC fertilisers Regulation (to be revised in the short term) and Plant Protection Products Regulation in the EU, in order to identify possible overlaps.

With this aim, scientific databases were searched for peer-reviewed articles describing plant biostimulants. It appears that the use of the term "biostimulant" is increasing rapidly in the scientific databases. Despite the adoption of the concept by the scientific community, no widely-accepted definition could be found. From the more than 250 articles using the word biostimulant in their titles or abstracts, eight categories of biostimulants were proposed : (1) humic substances, (2) complex organic materials, (3) beneficial chemical elements, (4) inorganic salts (such as phosphite), (5) seaweed extracts, (6) chitin and chitosan derivatives, (7) antitranspirants, (8) free amino acids and other N-containing substances. Some overlap may exist between these categories, which were mainly defined for the systematic analysis of the known action mechanisms of biostimulants, as reported by the peer-reviewed articles. This list should not be regarded as exhaustive, nor should it be regarded as a closed list for regulatory purposes, as the identification and agricultural use of new biostimulants may be expected in a near future. Furthermore, within the limited scope of this contracted study, it was decided not to include microorganisms, but this does not preclude their recognition as biostimulants in the future.

On the basis of this scientific review, the following definition is proposed and each of its elements is justified : "*Plant biostimulants are substances and materials, with the exception of nutrients and pesticides, which, when applied to plant, seeds or growing substrates in specific formulations, have the capacity to modify physiological processes of plants in a way that provides potential benefits to growth, development and/or stress response* ". The term "plant conditioners" is proposed as a synonym, which gives account of the capacity of biostimulants to enhance nutrition efficiency and/or stress response.

Finally, the proposed definition was confronted with the current definitions of Fertilisers and of Plant Protection Products in the EU law. It appears that the strict application of the existing definitions imposes to include biostimulants within Plant Protection Products. However, this situation is not satisfactory as it does not acknowledge the originality of biostimulants. This calls for a revision of the current legal definitions of Fertilisers and Plant Protection Products in the EU regulations.

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1. Objectives and methodology of the study

The objectives of this study are to evaluate the scientific robustness of the concept of plant biostimulants, to identify the substances and materials covered by the term biostimulant in the scientific databases, to propose a definition based on the modes of action described by the scientific literature, and, on this basis, to draw conclusions regarding the possible future status of biostimulants in the EU law.

With this aim, scientific databases were searched for peer-reviewed articles describing plant biostimulants. For practical reasons, it was agreed with Commission services to exclude microorganims from this first study, despite their occurrence in the literature on biostimulants. Consequently, this scientific survey is not exhaustive, nor should it be regarded as providing a closed list of biostimulants for regulatory purposes, as the identification and agricultural use of new biostimulants is expected in a near future. Synthetic hormones, hormone analogs and herbicide safeners were also removed from the list, due to their well established satus in the EU legislation. This is also justified by the fact that the term biostimulant appears to be marginally used for describing them.

The search results were analysed to assess the acceptance of the term biostimulants by the scientific community. The question of whether an existing definition imposed itself in this research field was addressed by examining the contents of these articles. Categories of biostimulants were identified based on the search results. For each category, the existing knowledge about the nature, origin and modes of action was summarized. This allowed to propose an original, overall definition of plant biostimulants. Finally, this definition was confronted with the existing definitions of Fertilisers and of Plant Protection Products in the EU law, and consequences for the future regulation of biostimulants were briefly discussed.

2. The concept of Biostimulant : bibliometric analysis

The scientific soundness of the concept of "biostimulant" was first analysed. Two approaches were followed. First, a bibliometric search was performed in scientific databases and the occurrence of the word "biostimulant" in peer-reviewed articles was analyzed. Second, scientific publications were searched for definitions of the term "biostimulant" and, in case of such definitions, their adoption by the other researchers active in the field was evaluated.

2.1. Occurrence of the term biostimulant in scientific databases.

Methods :

Database search used the SciVerse Scopus database and search algorithms provided by the University of Liège (Belgium).

As described by the service provider, Scopus is the largest abstract and citation database containing both peer-reviewed research literature and quality web sources. With over 19,000 titles from more than 5,000 international publishers, SciVerse Scopus offers the scientific community a comprehensive resource to support bibliographic and bibliometric analyses (SciVerse Scopus at a glance July 2011: more than 19,500 titles, including 18,500 peer-reviewed journals, 425 trade publications, 325 book series, 250 conference proceedings).

Results :

The Scopus database was searched using the query words "biostimulant(s)" or variants ("bio-stimulant", "biostimulation", etc.) in the titles, abstracts and keywords of the database entries. In order to restrict the search results to the articles in the plant domain, the word "plant" was added as additional search query (presence required in any field of the article). This allowed to remove papers which used the term biostimulants in remote fields like medicine, cosmetics, etc. It also appeared that the word biostimulant is used in articles related to industrial biotechnology and dealing with bioremediation activities of microorganisms. Most of these articles could also be removed from the search results when adding the word "plant" to the search query.

Figure 1 presents the results, displaying the number of scientific documents along the years. A limited number of records of plant biostimulants appears from the mid seventies, but a rapid increase of the number on documents is observed from the mid-nineties.

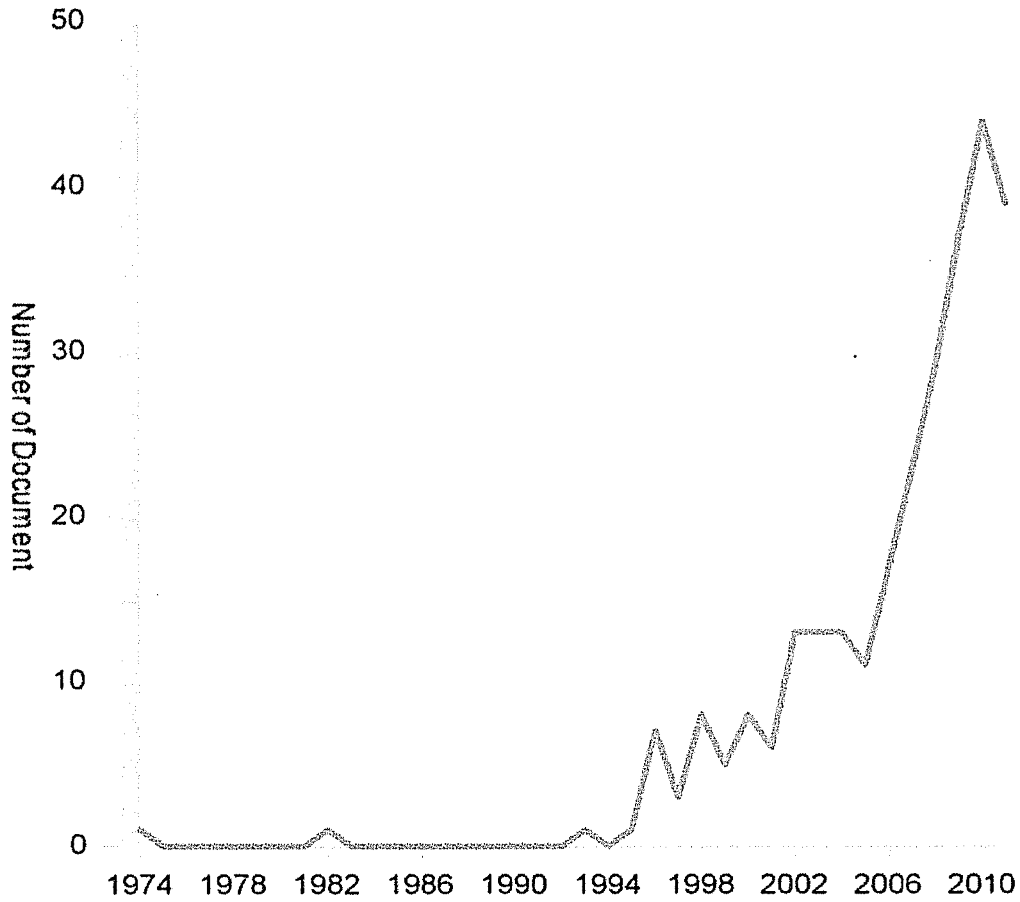

Figure 1. Number of peer-reviewed articles containing the term « biostimulant » (and variants) in their titles and/or abstracts in the Scopus database over time. Documents were filtered for the presence of the word « plant » in any search fields of the documents, to restrict the search to plant biostimulants.

2.2. Is there a consensus definition of plant biostimulants in the scientific literature ?

It appears that the first definition of biostimulants in the agricultural and horticultural fields was proposed, not by scientific papers, but by a web source of information for grounds maintenance professionals, called *Ground Maintenance* (http://grounds-mag.com). In 1997, in this web journal, 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 implicitely wanted to discriminate biostimulants from nutrients and soil amendments, which also promote plant growth but are clearly applied in larger quantities. The biostimulants mentioned by this web article are humic acids and seaweed extracts and their action on plants is proposed to be essentially hormonal.

Surprisingly, peer-reviewed papers published later by these authors on the same or similar research did not necessarily use the term biostimulant. For instance a paper by

Zhang and Schmidt in the year 2000 describing the use of humic acis and seaweed extracts for increasing drought tolerance of turfgrass did not use the term biostimulant at all (Zhang & Schmidt 2000). The paper in fact focused on the hormone-like activities of these compounds and the term "Hormone-containing products" was used instead of biostimulants to define this category of compounds[1]. This is in line with the initial assumption about biostimulants action in plants, as expressed in the 1997 web article (Zhang and Schmidt 1997) : "*Many important benefits of biostimulants are rooted in their ability to influence hormonal activity*". This pioneering review on biostimulants claimed various effects, pointing to antioxydants as a second line of action mechanisms, explaining the improved stress response of biostimulated plants.

In later peer-reviewed papers of Zhang and Schmidt, the term "biostimulants" and "metabolic enhancers" both appeared (Zhang et al. 2003a, 2003b). The adoption of the definition of Zhang and Schmidt 1997 by the research community was likely precluded by the lack of scientific notoriety of the web journal. However, the definition was later mentioned 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. Kauffmann 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 bibliographic survey of this report indicates that later papers expanded the use of the word "biostimulants" to other compounds (including *e.g.* amino-acids, phosphites, silica, etc.) as well as other modes of actions (e.g film-forming antitranspirant, gene regulators, osmolytes, etc.).

In conclusion, the scientific literature failed to reach a consensus on the definition of biostimulants. This did not prevent, and probably even promoted, the use of the term for a wide range of compounds, acting through diverse mechanisms with the final result of promoting plant growth and stress response, under defined conditions.

The final word could acknowledge the pioneering work of Zhang and Schmidt (1997), by quoting their words :

"Biostimulants are defined by what they do more than by what they are, since the category includes a diversity of substances. As the name suggests, they stimulate growth, but they do much more. Stress tolerance is perhaps the most important benefit of biostimulants."

As a milestone in the scientific acknowledgement of biostimulants, a first international scientific congress will be specifically dedicated to Biostimulants in a near future. The

[1] This could be due to the existing regulation in the US, as the US EPA registration process does not have a category of biostimulants , whilst a category of « hormone-containing products » does exist.

"*First World Congress on the use of Biostimulants in agriculture*" will be organized by public researchers and corporate representatives in Strasbourg, in next November 2012. Such initiative should be determinant in defining the future acceptance of the concept of biostimulants.

2.3. Conclusions of the literature review :

1- The word Biostimulant is used by scientific papers and the number of peer-reviewed articles using this term is increasing rapidly.
2- No definition imposed itself in the scientific literature, despite some attemps to clarify the concept.
3- The usefulness on the concept of biostimulants could reside in its capacity to describe a diversity of effects achieved by a diversity of substances, all resulting in plant growth promotion and stress response enhancement. This status of "open concept" (not locked by a strict definition) likely contributes to the increasing success of the term biostimulants.

3. Overview of the major classes of biostimulants

As a preliminary step of this report, a scientific review was performed, searching the SciVerse Scopus database (see above for a description of the database) with the word "biostimulant" (and variants : bio-stimulant, biostimulation, etc.) as search query, than limiting the list by adding "plant" in all search fields. The downloaded reference list (over 250 references) was manually edited, removing non relevant hits (*e.g.* physical biostimulation by irradiation). From the reading of these papers and the consultation of their bibliographies, articles were added to the reference list, exceding at the end 400 research articles on the subject. The titles and abstracts of all these references have been compiled in a separate document, in a searchable format, and annexed to this report.

This survey allowed to identify the compounds recorded as biostimulants. For the later description of biostimulants, they were classified as :

- Humic substances,
- Complex Organic materials,
- Beneficial chemical elements,
- Inorganic salts, including phosphite,
- Seaweed extracts,
- Chitin and chitosan derivatives,
- Antitranspirants,
- Free Amino acids and other N-containing substances.

This calls for several remarks :

1- These categories are not mutually exclusive and should not be strictly opposed to one another. For example « Complex organic materials » may contain « humic substances » as well as « free amino acids », but the three groups will be treated in separate sections. The justification is that specific formulations are described in the literature and the scientific data may specifically address one or the other group of compounds. As a second example, « antitranspirants » include film-forming compounds, but also « chitosan », which will be treated in a separate section due to its unique effects on the plant immune system.
2- Several major classes of compounds and agents were not covered, despite the fact that the word biostimulant has been used to describe them. In particular, microorganisms are occasionally described as biostimulants (in composts, waste-derived products, etc,), but deserve a special bibliographic analysis, out of the scope of this review. Plant hormones or hormone analogs, as well as « herbicide safeners » which enhance specific plant metabolisms, have « plant biostimulation » activites but they will not be considered by this review, considering that they are well-established categories of compounds, both in terms of scientific knowledge of their actions and of recognition of their legal status. As such, they are clearly out of the « grey zone » typical of the major biostimulants.

Each of the above categories is described in the following sections of this report. Their chemical nature, their sources and their modes of action accounting for their biostimulation activity are described. The main bibliographic references are quoted, with a view to identify reviews and recent articles providing additional access to the scientific literature. A « scientific review » has been provided to the Commission services in addition to this report, under the format of a list of references in the alphabetic order of their authors, where most of the abstracts of relevant articles can be found.

3.1. Humic substances

What they are.

Humic substances (HS) are natural substances belonging to the soil organic matter and resulting from the decomposition of dead cell materials and from the metabolic activity of soil microbes using these substrates. HS are collections of heterogeneous compounds, originally classified according to their molecular weights and solubility into humins, humic acids and fulvic acids, but with loosely defined boundaries and complex molecular constituents. Later definition of HS (reviewed in Piccolo, 2001) has payed increased attention to their dynamics in the soil, pointing to their association into supramolecular structures with larger apparent molecular sizes and stabilized by weak, non covalent chemical bonds (Nardi et al. 2007). As a consequence, plant root exudates containing organic acids and protons extruded from the cells have the capacity to disrupt these chemical bonds and to impact the formation and dissociation of humic macrostructures. This role was overlooked in the past and highlights a complex and mutual influence between plants roots and the soil organic matter.

Where they come from.

Humic substances with interest for the management of soil fertility have different origins. They can be extracted from humified organic matter from soils (*e.g.* from peat or volcanic soil, Nardi et al. 2007), or waste materials (*e.g.* sewage sludge, Ayuso et al, 1996a, 1996b). In broad terms, the soil organic matter contains a non-HS fraction - including molecular materials with chemical equivalents in living cells, *i.e.* amino acids, lignin, lipids, nucleic acids, etc. - and a HS fraction consisting of transformed, heterogeneous, high-molecular mass material with no strict chemical counterpart in living cells (for review, see Piccolo, 2001). Taken into account the extensive variability of soils, including their microbial and plant communities, and of the climatic conditions influencing the slow processes of HS formation, natural HS extracted from soils are expected to be chemically much variable. As a first step in HS standardization, HS can also be extracted from compost and vermicompost, where the starting organic subtrates, the bacterial communities and the environmental conditions may be monitored and controlled to some extent (Eyheraguibel et al. 2008). A significant achievement in the standardization of HS comes from the use of leonardite, a mineraloid substance extracted from lignite deposits. It is an oxidation product of lignite, easily soluble in alkaline solutions, providing a range of non-homogeneous compounds. Finally, 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" (Eyheraguibel et al. 2008). These are proposed as substitute for natural HS, based on their similarity in composition, structure and properties.

What they do.

HS act on soils and on plants.

Soil organic matter, including HS, is recognized as a major determinant of soil fertility, which covers physical, physico-chemical, chemical, and biological activities (Bronick 2005). Thus, HS contribute to the formation and stability of soil aggregates, hence to soil aeration and hydration (physical effects), to nutrient adsorption and availability, acting on the CEC ("Cation Exchange Capacity") of the soil (physico-chemical effects), to chemical reactions , *e.g.* producing secondary carbonates contributing to soil carbon sequestration (chemical effecs), to bacterial respiration (biological effects). Among their essential roles in soils, humic acids are adsorbed onto clay particles via polyvalent cations, making them specially effective in overcoming clay dispersion (Bronick 2005) .

Many effects of HS on soil fertility are mediated by mineral and organic compounds with which they interact and are dependent on their environment. For instance, HS may act on phosphate acquisition by plants by keeping P in solution but this beneficial effect will depend on soil pH and calcium concentration (Delgado et al. 2002). As another example, HS may have opposite effects with regard to water retention in the soil : HS may positively contribute soil porosity and to water retention in soils when interacting with clay particles and bound polycations, but addition of pure humic acids to artificial sandy soil may promote water loss, by forming water-repelling coatings on small soil particles (Van Dyke et al. 2009).

As regards nutrient acquisition and the resulting plant-growth promotion, the effects of HS are dependent on the dose of HS applied and on the nutrient (Ayuso et al. 1996a; 1996b; Kirn et al. 2010; Morard et al. 2010; Sánchez-Sánchez et al. 2006). Both positive and negative impacts on plant growth are described and application modes should be well defined to harvest the possible benefits.

Humic substances have direct impacts on the plant physiology. By direct effects, we mean effects that are not mediated by soil characteristics and nutrients availability but involve the regulation of cellular activities, like metabolic changes, altered gene expressions and hormonal actions. As indicated before, humic substances should be regarded as macromolecular structures which can be disrupted in the rhizosphere by components of root exudates, leading to soluble humic molecules (Canellas et al. 2008). These molecules are proposed to diffuse to and within root tissues, where they can interact with cell membranes and modify metabolic activities in the apoplasm (the phase formed by all continuous extracellular spaces). Stimulation of ATP-dependent proton-pumps (essential for nutrient import and for cell enlargement), of mitochondrial respiration (providing the energy carrier ATP), of invertase activities (delivering usable carbon substrates to mitochondrial respiration) has been described, indicating how soluble HS may promote root and plant growth. (Canellas et al. 2002; Canellas et al. , 2011; Ertani et al. 2011; Nardi et al. 2007; Schiavon et al. 2010; Vaughan 1979a, 1979b; 1984; Ord & Vaughan 1978).

Evidence for hormonal effects has been provided by using standard bioassays and, more recently, by molecular biology tools, including cellular reporters of auxin-responsive genes and gene-targeted mutants (Cacco & Dell'Agnola, 1984; Canellas et al. 2011, Dobbss et al. 2010; Masciandaro et al. 2002; Muscolo et al. 2007; Nardi et al. 2002; Schiavon et al. 2010). The exact origin of the hormone(-like) activities has been debated : whether they are supported by functional groups of the humic molecule, involve the release of entrapped hormone-like compounds from soil humic complexes, or even indirect effects due to some enhanced microbial activity. However, the effects of purified humic acids and of foliar applications of HS indicate that HS do have hormone-like activities in the absence of soil and of rhizobacteria. (Karakurt et al. 2009; Nardi et al. 2002; Ozdamar et al. 2011). This does not rule out additional sources of hormones from the soil environment.

The proposed biostimulation activity of HS also refers to biotic and abiotic 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, providing strong argument for a stress response modulation by HS (Schiavon et al. 2010). Limited information has also been published on the stimulation of antioxidant enzymes, which are general stress protectors, by HS-containing formulations (de Vasconcelos et al. 2009)

Overview : descriptors of this category

1. Existence of plant analogs :	No
2. Action inside the plant :	Yes
3. Action outside the plant (soil, leaf surface) :	Yes (most significantly)
4. Physical or physico-chemical (osmolytes) effects in/on the plant:	No
5. Metabolic effects (including antioxydants) :	Yes
6. Hormonal (hormone-like) effects :	Yes
7. Physiological effects on nutrition efficiency :	Yes
8. Physiological effects on abiotic stress response	Yes
9. Physiological effects on biotic stress response	Yes

3.2. Complex Organic materials

What they are.

Complex organic materials are obtained from composts, manure, sewage sludge extracts, agro-industrial and urban waste products. They can be applied on soils and on plants, with the aim to increase soil organic matter, to improve physico-chemical characteristics of soils, to provide macro- and micronutrients, to promote rhizobacterial activity, nutrient cycling and nutrient use efficiency, to control soil-borne pathogens, to enhance the degradation of pesticide residues and of xenobiotics. The promotion of plant growth and of crop yield in defined conditions of use explains why the term biostimulant is used to refer to these organic materials.

Seaweed extracts and amino-acid preparations, though they could also be defined as complex organic materials, will be treated in separate sections.

Many of these organic mixtures contain humic substances accounting for part of their activity, but other mineral, organic and microbiological components also play significant roles.

Considering the wide range of sources, treatments and formulations of these organic mixtures, any generalization is delicate and case-by-case examination should be recommanded. Examples of such categories of products and of their agricultural uses can be found in Ayuso et al. (1996), Eyheraguibel et al. (2008), Kvasauskas & Baltrenas, (2009), Lal et al. (2010), Lakhdar et al. (2010), Larkin (2008), Lopez-Mondejar et al. (2010), Pecha et al. (2011), Vidal-Beaudet et al. (2009), Sayara et al. (2011), Schiavon et al. (2008) and their cited references.

Where they come from.

The starting organic materials are very diverse : by-products of agro-industrial processes, sewage sludge from wastewater treatment plants and from the agro-food industry, manure, compost extracts from urban waste, fermentation tanks residues, etc. Nitrogen-rich sources are sometimes used, including protein hydrolysates from leather (Montemurro et al. 2010; Pecha et al. 2011) and from plant tissues (*e.g.* from alfalfa, Schiavon et al. 2008). Both aerobic and anaerobic microbial transformation of the organic materials can be achieved (see Larkin 2008 and Kvasauskas & Baltrenas 2009 for respective examples). In some applications, bacteria and fungi are added or stimulated, for the biocontrol of pathogens and for nutrient use improvement (*i.e.* by the use of mycorhizal fungi). The role of microorganisms in biostimulants is, by convention with the contracting authority, considered as out of the scope of this scientific review.

What they do.

The modes of actions cover the many additive and synergic effects of the mineral, organic and microbiological components of these complex materials. Direct and indirect, immediate and delayed effects are all described in the quoted scientific litterature (see above), on soil properties and on plant physiology. Effects on the physiology of the plant include metabolic changes and hormonal effects. Regarding plant growth and crop yield, positive and negative effects may occur, depending on the applied dose and on possible contaminants (*e.g.* heavy metal contaminants in sewage sludge). They depend on the biostimulant preparation and on the target plants and soils, taking into account the receiving environments. Their efficacy and safety should be assessed on a case-by-case basis.

Overview : descriptors of this category :

1. Existence of plant analogs	Yes
2. Action inside the plant	Yes
3. Action outside the plant (soil, leaf surface)	Yes
4. Physical or physico-chemical (osmolytes) effects	Yes
5. Metabolic effects (including antioxydants)	Yes
6. Hormonal (hormone-like) effects	Yes
7. Physiological effects on nutrition efficiency	Yes
8. Physiological effects on abiotic stress response	Yes
9. Physiological effects on biotic stress response	Yes

3.3. Beneficial chemical elements

What they are.

Chemical elements that promote growth and may be essential to particular taxa but are not required by all plants are called beneficial elements (reviewed in Pilon-Smits et al. 2009). The five main beneficial elements are Al, Co, Na, Se and Si. They are present in soils and in plants as different mineral species, and both soluble and insoluble forms are found, depending on the pH and on interactions with various ions and organic molecules. The definition of beneficiel elements must refer to the particular plant species where they exert some beneficial function. These beneficial functions can be constitutive, like the strengthening of cell walls by deposits of amorphous silica ($SiO_2.nH_2O$) in graminaceaous species, 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.

These elements are also of importance for human and animal physiology. For instance, Selenium is essential in antioxydant response in humans. "Biofortification" of crops uses traditional and molecular plant breeding, but may also take advantage of crop fertilizing techniques, for increasing the content and availability of essential and beneficial elements in human diets (Hirschi 2008; Nestel et al. 2006)

Where they come from.

These elements may be present as abundant (*e.g.* Si, Na) or rare (*e.g.* Se, Co) chemicals in the environment. They will be present in trace or larger amouns in a wide range of fertilisers. Their availability in the soil may strongly depend on the pH and on root exudates, like for Al and Co. When they are intentionally applied to plants as biostimulants, various sources are identified. Taking the example of Si, which receives much interest as plant biostimulant, it may be obtained as calcium silicates from industrial slag by-product (Ca_2SiO_4), from geological deposit of wollastonite ($CaSiO_3$), but also form ashes of agricultural by-products and other sources (Reynolds et al. 2009; Savant et al. 1999). Some irrigation waters are adequate suppliers of silica (Savant et al. 1999).

Is is assumed that the bioactivity of some complex biostimulants, like compost teas and plant extracts, involves the physiological functions of the contained minerals, of which beneficial elements might play a significant role.

What they do.

We recommand to the reader the review of Pilon-Smith 2009 for an overview on the physiological functions of beneficial elements. The special and important case of silicon is developed in Savant et al. (1999), Reynolds et al. (2009), Ma (2011). In accordance with the definition of beneficial elements above, their beneficial functions and the plant strengthening effects aimed by fertilizing practices will strongly depend on the crop species and on the environmental conditions.

Thus, the many effects reported by the scientific litterature include cell wall rigidification, herbivore deterring, 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, antioxydant protection, interactions with symbionts, pathogen and herbivore response, protection against heavy metals toxicity, plant hormone synthesis and signalling (Pilon-Smits et al. 2009)

Overview : descriptors of this category

1. Existence of plant analogs	Yes
2. Action inside the plant	Yes
3. Action outside the plant (soil, leaf surface)	Yes
4. Physical or physico-chemical (osmolytes) effects	Yes
5. Metabolic effects (including antioxydants)	Yes
6. Hormonal (hormone-like) effects	No
7. Physiological effects on nutrition efficiency	Yes
8. Physiological effects on abiotic stress response	Yes
9. Physiological effects on biotic stress response	Yes

3.4. Inorganic salts, including phosphite

What they are.

Many organic salts, including phosphites and phosphates, but also bicarbonates, sulphates, nitrates, provide protection against fungi, which may involve direct fungicidal action or indirect protection by stimulating plant defenses (reviewed in Deliopoulos et al. 2010). Their action on the physiology of the plant, on stress response and on yield explains why these inorganic compounds are sometimes referred to as biostimulants.

The term phosphite is used to refer to the salts of phosphorous acid (H_3PO_3) whilst phosphite esters are designated as phosphonates, which include ethyl-and methyl phosphonates. Fosetyl aluminium is a phosphonate, precisely aluminium tris-*O*-ethyl phosphonate. In practice, the term phosphite is sometimes used for designating both phosphite salts and phosphite esters. They are clearly distinct from phosphates, which are salts of phosphoric acid (H_3PO_4) and constitute the main sources of P used by plant nutrition. P fertilisers used in agriculture are phosphates.

Where they come from.

These inorganic salts are provided by traditional processes of inorganic chemistry and by extraction from geological deposits. Case-by-case description is needed and is out of the scope of this review.

What they do.

The function of phosphite in plants and the question of whether it is a source of P for plant nutrition has been the subject of controversy (reviewed in Thao & Yamakawa 2009). Plants take up and use phosphate in their metabolism, not phosphite. Oxidation of phosphite into phosphate could theoretically deliver usable P in the plant, but such oxidation is not documented in plant cells. Limited evidence is available regarding the capacity of soil bacteria to achieve this oxidation (see references cited in Thao & Yamakawa 2009), but the process is slow and there is no evidence for such conversion by bacteria living on, or within, plant organs that would support their capacity to utilize phosphite as a P fertiliser. However phosphite has marked effects on the plant physiology, providing protection against fungi (belonging to the Oomycetes, including *Phytophthora* species, causing major plant diseases) and Fosetyl aluminium has become a popular systemic fungicide. Phosphite has an influence on P nutrition, but recent insight into the biochemical and genetical mechanisms of phosphite action indicates that phosphite behaves as a phosphate analog, "mimicking" phosphate in plant and yeast cells but without making its P available for cell metabolism. As a consequence, phosphite represses the so-called "phosphate-starvation response" of plants and can make the plant less effective in phosphate sensing, uptake and metabolization, leading to detrimental effects in phosphate-limited environments (da Cruz et al. 2011; Carswell et al. 1996; Carswell et al. 1997; Förster et al. 1998; Schroetter et al. 2006; Thao & Yamakawa 2010; Varadarajan et al. 2002). Thus, phosphite down-regulates phosphate transporters, enzymes involved in the extracellular P mobilization (*e.g.* acid phosphatase) and various proteins and metabolites associated with the coordinated plant's response to phosphate deficiency. In yeast, where the genes controlling the Pi-starvation response are very well known, the analysis of mutants treated by phosphite identified target genes belonging to the metabolic network of phosphate sensing and use (McDoldan et al. 2001, cited in Thao & Yamaka 2009).

Under adequate phosphate supply, phosphite may contribute to yield and product quality (Lobato et al. 2011; Lovatt 1999; Lovatt & Mikkelsen 2006), which can be explained by the effective protection against ubiquitous fungi and by complex metabolic effects likely associated to phosphate regulation. Cellular phosphate is not only a supplier of P and a building block of many biological molecules (*e.g.* nucleic acids), but it plays essential functions in the cell, including in the energy processing networks of photosynthesis and respiration.

As regards the other inorganic compounds mentioned - phosphates, silicates, nitrates etc.-, their contribution to the protection of plants against fungal diseases is explained by both direct fungicidal effects, like inhibition of sporulation and hyphal growth, and by the stimulation of plant defense. These functions are extensively reviewed by Deliopoulos et al. (2010).

Overview : descriptors of this category

1. Existence of plant analogs :	Yes (e.g. phoshate) or No (e.g. phosphite)
2. Action inside the plant :	Yes
3. Action outside the plant (soil, leaf surface) :	Yes
4. Physical or physico-chemical (osmolytes) effects :	Yes
5. Metabolic effects (including antioxydants) :	Yes
6. Hormonal (hormone-like) effects :	No
7. Physiological effects on nutrition efficiency :	Yes (NB : negative impact of phosphite on P nutrition described in certain conditions)
8. Physiological effects on abiotic stress response :	Yes
9. Physiological effects on biotic stress response :	Yes

3.5. Seaweed extracts

What they are.

Seaweeds constitute a vast group of species which are classified into different phylums, including brown, red and green macroalgae. Molecular systematics has demonstrated their early divergence in the evolution of photosynthetic organisms. They should be regarded as well separate taxonomic entities and this should be born in mind when attempting a general description of their biochemical and functional characteristics.

Seaweeds and seaweed extracts are used as biofertilisers, soil conditioners and biostimulants. In fact, the use of fresh seaweeds as source of organic matter and as fertiliser is very old in agriculture. Only recently, chemical analysis, immuno- and bioassays have identified the many compounds which contribute to the plant growth promotion effects (reviewed in Khan et al. 2009 and in Craigie 2011). Micro- and macronutrients, special polysaccharides (like alginates, laminarin and carragheenans), sterols, N-containing compounds like betaines, and hormones, are all recognized as bioactive components. Many of these compounds are indeed unique to their algal source, explaining the increasing interest of the scientific community and of the industry for these groups of species.

Where they come from.

Many processed products have been developed, from very crude extracts to highly purified products, depending on the scope of application and targeted compounds. Alginate is an example of purified polymer from brown algea with many and diverse applications, in agriculture, food and pharmaceutical industry. Product names of the more thant 20 seaweed products used as plant growth stimulant are reviewed in Khan et al. (2009).

What they do.

Seaweeds act on soil and on plants (reviewed in Khan et al. 2009, Craigie 2011; Craigie et al. 2008). They can be applied on soils, in hydroponic solutions or as foliar applications.

In soils, the special properties of their polysaccharides contribute to gel formation, water retention and soil aeration. Their 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 now viewed as major causes of biostimulation activity on crop plants. Cytokinins and auxins are the main hormones identified but abscisic acid, gibberellins and other classes of compounds with hormone-like ativities, like sterols and polyamines have also been described (reviewed in Craigie 2011). These hormonal functions have been demonstrated by bioassays (where seaweeds extracts are used in standardized biological tests in parallel with purified hormones) and by the immunological and chemical identification of compounds related to known plant hormones.

Overview : descriptors of this category

1. Existence of plant analogs	Yes (e.g. auxins) or No (e.g. alginates)
2. Action inside the plant	Yes
3. Action outside the plant (soil, leaf surface)	Yes
4. Physical or physico-chemical (osmolytes) effects	Yes
5. Metabolic effects (including antioxydants)	Yes

6. Hormonal (hormone-like) effects	Yes
7. Physiological effects on nutrition efficiency	Yes
8. Physiological effects on abiotic stress response	Yes
9. Physiological effects on biotic stress response	Yes

3.6. Chitin and chitosan derivatives

What they are.

Chitin and its deacetylated forms chitosan are bioactive biopolymers from which many derivatives are produced by hydrolysis and chemical modification, for increasing their water solubility and bioactivity (for review : El Hadrami et al. 2010; Yin et al. 2010). They can be collectively referred to as chitoologosaccharides.

Where they come from.

Chitin is natural polymer found in all marine and terrestrial ecosystems, where it constitutes the exoskeleton of insects, crustaeans and the cell wall of true fungi (*Eumycota*). Crustaceans and fungi are the main source of the industrial extraction of chitin, which is the starting point of the many chitooligosaccharides obtained by size-reduction and chemical modifications, depending on the industrial applications. Chitoologosaccharides are used as food and feed additives, antimicrobial agents in medecine, in cosmetics, as plant antitranspirants and growth stimulators, plant protection products, etc.

What they do.

Chitin derivatives can have actions outside living cells, as polycationic and lipid-binding molecules. This leads to applications for water cleaning, but the formation of chitosan films on the waxy surface of plant leaves prompts their use as antitranspirant.

The actions within the plant are manyfold and have been recently reviewed by El Hadrami et al. (2010) and Yin et al. (2010). The main function is in plant protection against virures, bacteria, fungi and insects, via the activation of host defense genes and the development of so-called immune response. Plants do not produce antibodies and their immunity is of a clearly different nature compared with animals, but they can express a range of protective compounds including secondary metabolites, chelatants, cell wall hydrolases, enzyme inhibitors and sugar polymers acting as physical barriers.

Recent insight into the molecular modes of action points to the existence of common sensing and signalling pathways of microbial cell wall fragments, in both beneficial and pathogenic interactions (Hamel & Beaudoin 2010; Silipo et al. 2010). Chitooligosaccharides are recognized by the same receptors and trigger the same signaling cascade and expression of defense genes as the pathogens cell wall fragments functioning as elicitors of plant defenses. Transcriptomic profiling of the genes up- and downregulated by chitosan has been recently achieved in the model plant *Arabidopsis thaliana* (Povero et al. 2011).

The plant defense responses elicited by the chitooligosaccharides include lignification, callose plug formation, synthesis of proteinase inhibitors and of cell wall hydrolases (chitinases and glucanases), of phytoalexins, generation of active oxygen species, cellular responses like ion flux variations and membrane depolarization, cytosol acidification and changes in protein phosphorylation (see El Hadrami et al. 2010; Amborabé et al. 2008 and references therein). Film formation by chitosan poly- and oligomers at the surface of the leaf is assumed to provide additional protection against air-borne pathogens.

Overview : descriptors of this category

1. Existence of plant analogs	No
2. Action inside the plant	Yes
3. Action outside the plant (soil, leaf surface)	Yes
4. Physical or physico-chemical (osmolytes) effects	Yes
5. Metabolic effects (including antioxydants)	Yes
6. Hormonal (hormone-like) effects	No
7. Physiological effects on nutrition efficiency	Yes
8. Physiological effects on abiotic stress response	Yes
9. Physiological effects on biotic stress response	Yes

3.7. Antitranspirants

What they are.

As the term antitranspirant has been coined to indicate the overall effect on the plant, the chemical compounds and underlying mechanisms can be, and in fact are, very diverse. Some of the compounds have physical effects at the surface and/or within the plant organs, others are regulators of the leaves openings diffusing water vapor, called stomata.

The film-forming antitranspirants are obtained from mineral compounds (*e.g.* kaolin), synthetic polymers (*e.g.* polyacrylamide) and natural polymers (*e.g.* chitosan).

Metabolic antitranspirants include stomatal regulators, acting on the complex hormonal control of the highly specialized cells bordering the stomatal pore (called guard cells).

More information on the compounds and corresponding references are given below, related to their the modes of actions.

Where they come from.

The sources are very diverse, just as as the compounds are. Further information on the origin (and commercial suppliers) of the antitranspirants can be found in the cited literature.

The film-forming antitranspirants are : synthetic compounds (di-1-p-menthene or pinolene, naphtalene, polyacrylamide, etc. reviewed in Percival & Boyle 2009), inorganic compounds (based on kaolin clay and other silicates, Cantore et al. 2009; del Amor et al. 2010), on natural biopolymers (chitosan, Bittelli et al. 2001).

What they do.

Some film-forming compounds, like kaolin clay and chitosan increase leaf surface reflectance, reducing absorption of radiant energy (heat), lowering leaf temperature, reducing water evaporation within the leaf and its diffusion to the surrounding atmosphere (referred to as transpiration). Stomata are the main route of water vapor export during plant growth. When emulsions of the synthetic compounds are sprayed on leaf surfaces, they form thin films and limit gas exchange by increasing stomatal resistance to the diffusion of water vapor.

As both carbon dioxide and water vapor circulate via the same stomatal route between the leaf and the outer atmosphere (with opposite directions during active photosynthesis), any stomatal limitation of water vapor loss will also lead to a reduction in carbon dioxide diffusion to leaf tissues. The ratio between the two determines "water use efficiency", a critical parameter in crop ecophysiology. By acting on leaf resistance to vapor loss, antitranspirants may improve water use efficiency and yield in water-limited conditions, but their use must be adapted to the crop physiology and to the field conditions, in order to obtain benefits and avoid negative impacts on yield. The "biostimulation" effect is thus not achieved, and even achievable, in any conditions (Evans & Sadler 2008).

Other inorganic compounds, like soluble silicates sprayed on leaves may reduce transpiration (Savant et al. 1999). Diffusion of wator vapor within the leaves may also be influenced by insoluble silica and it has recently be realized that these effects may be of significance for plant ecology and species distribution as a response to climate aridity (Cooke & Leishman 2011).

Film formation not only changes water use efficiency, but also interferes with pathogen establishment and growth on the surface of the leaf, which is a major aim of their agricultural use (Percival & Boyle 2009; Walters 2006).

Other antitranspirants are physiological regulators of stomatal opening, like the hormone abscisic acid (ABA) and its analogs (see Goreta et al. 2007 and ref. therein). Inhibitors of ethylene biosynthesis (*e.g.* aminoethoxyvinylglycine) have been used with the aim to counteract its antagonistic effect towards ABA and its senescence-promotive effect under stress conditions, with variable success (Goreta et al. 2007 and ref. therein).

Chitosan works as both film-forming compound and physiological regulator of stomata via the ABA-dependent pathway (Bittelli et al. 2001 and references therein).

Overview : descriptors of this category

1. Existence of plant analogs	Yes (e.g. ABA analogs) or No (di-1-p-menthene)
2. Action inside the plant	Yes
3. Action outside the plant (soil, leaf surface)	Yes
4. Physical or physico-chemical (osmolytes) effects	Yes
5. Metabolic effects (including antioxydants)	Yes
6. Hormonal (hormone-like) effects	Yes (e.g. ABA analogs) or No (film-forming compounds)
7. Physiological effects on nutrition efficiency	Yes
8. Physiological effects on abiotic stress response	Yes
9. Physiological effects on biotic stress response	Yes

3.8. Free Amino acids and other N-containing substances

What they are.

Organic nitrogenous compounds include free amino acids (of protein and non-protein origins), peptides (of protein and non-protein origins), polyamines, betaines and related substances. These molecules belong to different plant and non-plant metabolisms and should be treated on a case-by-case basis. When applied to plants, they are mostly used as foliar applications, but soil applications and seed coating also exist with some of them. They are often used as mixtures and, as some formulations of amino-acids are produced by the hydrolysis of industrial by-products (from both animal, plant and microbial sources), their composition can be much variable. The biostimulants used in agriculture are mainly amino-acids mixtures. Useful reviews and scientific references will be found in Maini (2006), Parrado et al. (2007), Parrado et al. (2008), Schiavon et al. (2008) , Thomas et al. (2009), Vranova et al. (2011).

Where they come from.

Amino-acids and peptides mixtures can be obtained by chemical and enzymatic protein hydrolysis from agroindustrial by-products, from both plant sources (crop residues) and animal sources (*e.g.* collagen, epithelial tissues). They can also be produced by chemical synthesis.

Besides the 20 amino acids used as precursors in protein synthesis, some 250 "non-protein amino acids" are found in plants, in some botanical families in particular, and receive increasing attention with regard to their physiological and ecological roles (reviewed in Vranova et al. 2011). They are also found in soil and peat extracts. Glycinebetaine is a special case of non-protein amino acid.

Betaines and polyamines are organic N-containing compounds found not only in plants but in taxononomically remote organisms, including bacteria and mammals.

What they do.

Amino acids have been involved in multiple cellular and physiological processes. They also impact on plant nutrition by forming chelates and complexes with soil nutrients.

Amino acids applied to plants have effects on their metabolism, including nitrogen and carbon metabolisms, primary and secondary metabolisms (Schiavon et al. 2008, Maini 2006 and ref. therein). Most notably, several enzymes of the nitrate assimilation pathway – nitrate reduction and ammonium-to-amine conversion - are stimulated, and molecular analysis has indicated that this upregulation is achieved at the gene expression level, to a large extent. Enzymes of the Krebs cycle are also stimulated, which is expected in any situation of enhanced synthesis and recycling of amino acids. Taken together, these data provide support to the hypothesis that exogenously applied AAs have the capacity to enhance the nitrogen use efficiency of the plant. This in turn provides general stimulation to photosynthesis and plant growth.

Hormonal functions have also been ascribed to free amino acids and peptides, including auxin- and gibberellin-like activities (Schiavon et al. 2008).

Whether they result from the general enhancement of nitrogen and carbon metabolism or from specific effects on gene expression (or from both), free AAs and peptides have the capacity to stimulate plant defenses to biotic and abiotic stress (Schiavon et al. 2008, Maini 2006 and ref. therein). Furthermore N-containing metabolites, including AAs and especially proline and histidine have been associated with plant response to heavy metals, providing protective effects via different mechanims, like metal-binding, signalling and antioxidant defense (reviewed in Sharma & Dietz 2006).

N-containing compounds include the well-known stress response metabolites glycinebetaine and proline. Their accumulation in plant cells has been correlated with osmotic stress tolerance and their causal roles in this tolerance has been extensively investigated using transgenic plants (reviewed in Ashraf & Foolad 2007; Chen & Murata 2011). Accumulation of these metabolites was initially thought to protect the cells via an osmotic effect but more subtle roles involving physical interactions with photosynthetic components and with the gene expression machinery have been demonstrated (Chen & Murata 2011). Other abiotic stresses, like drought and salt stress, also trigger the synthesis of glycinebetaine and proline. These effects may be species- and environment-specific and the potential benefits of their application or induction in crops plants is still a matter of controversy (Ashraf & Foolad 2007).

Nitrogen metabolism is also of special importance in the production of secondary metabolites. These include non-protein amino acids (*e.g.* canavanine, ornithine, homoserine, etc.) and a vast number of alkaloids. These compounds have toxic effects on herbivores, but some of them also play roles in the plant's metabolism, in signalling, photodamage protection, membrane protection, morphogenesis, etc. (reviewed in Vranova et al. 2011). A feature of many of these secondary N-containing metabolites is that their production and function is highly specific to plant taxa and to the target organisms, which can be insects, microorganismes or plants. So-called allelopathic compounds, many of which are released in the soil by root exudation or plant decomposition include secondary N-containing compounds (Belz 2007; de Albuquerque et al. 2010).

Phytochelatins are metal-binding, thiol-containing peptides induced by heavy metal stress, active in the sequestring and detoxication of heavy metals in plant cells. These N-containing compounds stimulate plant growth when challenged by metal pollutants (Zitka et al. 2011).

Overview : descriptors of this category

1. Existence of plant analogs	Yes
2. Action inside the plant	Yes
3. Action outside the plant (soil, leaf surface)	Yes
4. Physical or physico-chemical (osmolytes) effects	Yes
5. Metabolic effects (including antioxydants)	Yes
6. Hormonal (hormone-like) effects	Yes
7. Physiological effects on nutrition efficiency	Yes
8. Physiological effects on abiotic stress response	Yes
9. Physiological effects on biotic stress response	Yes

4. Summary : how do plant biostimulants work ?

From the scientific data reported in the previous sections, several conclusions can be drawn :

1- All biostimulants show multiple modes of action, which may be additive and/or synergic ; the range of effects depends on the type of biostimulant.
2- Their common status of biostimulant is justified by their conditional, growth-promotion and/or stress response enhancement activities.
3- Nutrition efficiency and (a)biotic stress response capacity are the main physiological parameters accounting for plant biostimulation.
4- Biostimulants may act both inside and outside the plant organism.
5- Physical, metabolic, gene regulation and hormonal effects all participate to the activity of biostimulants.
6- Biostimulation is achieved by substances with contrasting chemical natures, from simple inorganic compounds to complex biomolecules.
7- Both general effects, *i.e.* applicable to a wide range of species, and specific effects, *i.e.* restricted to a few species, can be identified.
8- Biostimulation is conditional : the effects may depend on the nutritional status of the plant, the soil parameters, the pressure of pathogens, the abiotic stress conditions, etc.

No single, primary effect of biostimulants on the physiology of plants can be proposed, which would provide a clear-cut criterion for including substances and materials in the category of biostimulants. To make this statement more explicit, the literature data do not allow to propose some standard bioassay to experimentally assess the biostimulant activity of a new substance or material.

This does not preclude a common and scientifically sound definition of biostimulants, which is proposed in the next section.

5. Definition of biostimulants : a proposition derived from the literature review

In spite of the complexity and diversity of the modes of action of the biostimulants, and taking into account the deliberate exclusion of microorganisms from the scope of this scientific review, a definition is proposed by this report :

Plant biostimulants are substances and materials, with the exception of nutrients and pesticides, which, when applied to plants, seeds or growing substrates in specific formulations, have the capacity to modify physiological processes of plants in a way that provides potential benefits to growth, development and/or stress response.

Each component of this definition is commented :

"Plant biostimulants ..."

The term « biostimulant » is also used in the scientific literature to describe physiological effects on other organisms than plants, including microbes, animals and humans. The wording « plant biostimulants » clarifies the scope.

"... substances and materials ..." :

Biostimulants can be pure molecules (*e.g.* Calcium silicate, Ca_2SiO_4) or complex mixtures of substances (*e.g.* compost extracts). In the latter case, the composition can be known and standardized (*e.g.* chemically-synthesized amino-acids mixtures), or poorly characterized and variable (*e.g.* sewage sludge extracts).

"...with the exception of nutrients and pesticides ..."

Nutrients promote plant growth by the provision of chemical elements used by metabolic processes for the synthesis of biomolecules and for biomass production. Pesticides promote plant growth by reducing the detrimental effects of pathogens and pests on plant integrity and functionality. However these positive effects on plant growth and crop yield are distinct from "biostimulation", which refers to some action on plant physiological processes ("plant conditioning" will be proposed as a synonym of biostimulation at the end of this section). In order to make this distinction as clear as possible, nutrients and pesticides are explicitely excluded from the definition. It has to be clear that some biostimulants may have, at the same time, some pesticidal effect. For example, chitosan triggers plant immunity and, as such, is regarded as a biostimulant, but direct pesticidal effects on fungal pathogens are also observed. In such a case, the primary aim of the application should take priority over side-effects. As another example, amino-acids mixtures provide the plant with an additional nitrogen source, but this nutritional effect is not the aim of the application, whilst the gene-regulation and hormonal effects promoting plant growth should be considered as a priority for their categorization.

"... applied to plants, seeds or growing substrates ..."

No restriction in the mode of application of biostimulants is included in the definition. Soil application, foliar application, seed coating and root dressing are all possible.

"... in specific formulations ..."

Biostimulants may need fixed formulations for their bioactivity, by ensuring uptake by the plant, systemicity, and suitable additive or synergic effects between components. Even for simple compounds, the source and formulation may be important for bioactivity (*e.g.* the source and chemical form of silicon have a marked impact on its protective effect towards insect pests).

"... have the capacity to modify physiological processes of plants ..."

Biostimulants change how plants work, *i.e.* their physiology, and this may be described at any level of their organization : at the cellular level (*e.g.* activities of enzymes and of membrane transporters, regulation of gene expression), at the organ level (*e.g.* gas exchanges of the leaf, initiation of lateral roots by a pre-existing root), and at the whole-plant level (*e.g.* regulation of leaf transpiration by root-emitted signals). Plant growth itself may be regarded as a whole-plant physiological trait which integrates the many underlying processes of plant nutrition, photosynthesis, carbon allocation and coordinate organ development. Hormones are chemical mediators in the regulation and coordination of all physiological activities, allowing their integration during plant life.

Biostimulants must be recognized on the basis of their capacity to modify the physiology of the plant, which, as indicated, may be observed at any level of its functioning.

"...in a way that provides potential benefits ..."

The physiological effects of biostimulants depend on several parameters : the applied dose and formulation, the target plant species, the environmental conditions, including nutrients availability, etc. Any benefit is thus potential and conditional. When biostimulants activate stress response genes, the actual benefit will depend on the imposition of stress and its characteristics (intensity, duration, etc.).

"...benefits to growth, development, and/or stress response."

Growth expresses the rate of biomass increase over time, which results from photosynthetic carbon fixation and from the use of the photoassimilates for either biomass production or respiration. Development is a more qualitative manifestation of plant life, involving the sequential initiation and differentiation of organs, from seed to seed. Stress describes any state imposed by external factors which restrict plant growth and development and displace plant functioning from its optimum, or just from normality. Both biotic factors (pathogens, pests) and abiotic factors (nutrients deficiency, drought, heat, cold, etc.) may cause such a shift. Responses to stress conditions are manyfold but photosynthesis is most often threatened by adverse environments and many abiotic stress responses aim at protecting the photosynthetic apparatus. In particular, oxydative stress is a secondary stress caused by various adverse environments and antioxydants protect the photosynthetic machinery against harmful oxygen radicals and other active oxygen species. Several biostimulants have been shown to enhance the antioxydant capacity of plants, providing partial explanation for their improved vigour in adverse environments. As regards biotic factors, many plant genes are activated upon pathogen infection, many of them being rather non-specific to the trigger pathogen. When the term "immunity" is used for plants, it should be understood that, in contrast with humans, plants produce defensive compounds which are very different from the protein antibodies in mammals. Importantly, the stress compounds are rather a-specific, *i.e.* they are not specifically targeted to the invasive pathogen (like antibodies do with antigens), but have a broad spectrum of action. This is why chitooligosaccharides have the capacity to "immunize" plants towards viruses, which do not use any such saccharides for their packaging and infectivity.

Conclusion : what's special with biostimulants ?

To be recognized as a biostimulant, a substance or material must be shown to modify the physiology of plants, making them more efficient for using limited resources of their environment – like water and nutrients – or for protecting them against harmful agents, which can be reactive oxygen species induced by stressful environments, or pathogens or pests. What is instrumental in this definition is that the effect on growth and development must be due to the capacity of biostimulants to "condition" the plant for coping with adverse external factors. These adverse external factors may be mild and prolonged, like low phosphate availability in alkaline soils, or severe and sudden, like the inoculation of an aggressive plant virus by an aphid.

Should a synonym be proposed for plant biostimulants, we would favour "plant conditioners", which we think is in good accordance with the overall function of biostimulants. Biostimulation appears as a way of plant "conditioning" for the challenging of adverse, growth-limiting environments.

6. Biostimulants and the current EU law

The proposed definition stems from the scientific data analysed by this review and did not consider the provisions of the EU law regarding the substances and materials applied to agricultural and horticultural plants. The EU legislation describes two categories of products applied on plants - fertilisers and plant protection products – and the definitions of these two categories can now be compared with the definition of biostimulants proposed above.

6.1 : Are biostimulants "fertilisers" in the sense of the EU law ?

Fertilisers are defined by the *Regulation (EC) n° 2003/2003 of the European Parliament and of the Council of 13 October 2003 relating to fertilisers*, in Article 2 :

"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."

According to this Regulation, EC fertilisers are defined by the nutrient(s) they provide to the plant and by their chemical forms, including complexes and chelates (see Article 9 of the Regulation). Annex I of the Regulation lists the EC fertilisers identified by these criteria.

From this definition, we conclude that biostimulants should not be considered as fertilisers, because their main function is not to deliver nutrients to the plant.
Nevertheless, many biostimulants help plant nutrition, both in the soil and in the plant, by interacting with nutrients but also with plant components involved in the uptake and assimilation of nutrients.

For example :

1. Humic acids bind cations in the soil, increasing the Cation Exchange Capacity (CEC) of the soil and the availability of nutrients for plants.
2. Amino-acids mixtures stimulate enzymes of the nitrate assimilation pathway, like nitrate reductase which catalyses a rate-limiting step in nitrogen assimilation.
3. Seaweed extracts stimulate root growth and ion uptake capacity by auxin-like effects.

As a conclusion, **biostimulants may enhance the efficiency of nutrition**, but this effect is distinct from nutrient provision *sensu stricto*.

Not all biostimulants are enhancers of nutrition efficiency. For example, chitosan elicitors and pinolene-based antitranspirants may not be regarded as acting on nutrition, as a main characteristic.

6.2. Are biostimulants "plant protection products" in the sense of the EU law ?

Plant protection products are defined in the EU by the *Regulation (EC) n° 1107/2009 of the European Parliament and of the Council of 21 October 2009 concerning the placing of plant protection products on the market and repealing Council Directives 79/117/EEC and 91/414/EEC*, as follows :

"Article 2
Scope
1. 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) protecting plants or plant products against all harmful organisms or preventing the action of such organisms, unless the main purpose of these products is considered to be for reasons of hygiene rather than for the protection of plants or plant products;
(b) influencing the life processes of plants, such as substances influencing their growth, other than as a nutrient;
(c) preserving plant products, in so far as such substances or products are not subject to special Community provisions on preservatives;
(d) destroying undesired plants or parts of plants, except algae unless the products are applied on soil or water to protect plants;
(e) checking or preventing undesired growth of plants, except algae unless the products are applied on soil or water to protect plants.

These products are referred to as 'plant protection products'."

Considering that biostimulants were defined in this report as plant conditioners "having the capacity to modify physiological processes of plants in a way that provides potential benefit to (...) stress response", there is a clear overlap with part (b) of the EU definition. This part (b) is in fact very broad : it seems that, as soon as a product *"is intended to influence the life processes of plant"*, it should be covered by the Regulation.

The part (a) of the definition also appears to be very broad : *"protecting plants or plant products against all harmful organisms or preventing the action of such organisms"* may, in practice cover very different mechanisms and bioactive products. Any elicitor of plant defense genes, like chitooligosaccharides or phosphite, will fall under this definition, with no distinction from synthetic pesticides with direct pesticidal action against pathogens.

Many biostimulants will help protect the plant, but they do it differently from pesticidal compounds : they "help the plants help themselves". Biostimulants enhance the plant's defense capacity and this plant conditioning effect is also referred to as "plant strengthening" in the literature.

Not all biostimulants have protective effects against harmful organisms. Glycinebetaine, for example, is involved in abiotic stress tolerance but its contribution to resist pathogens and pests, is, at best, very limited.

6.3. Options for the future

Although the strict implementation of the legal definitions of fertilisers and of plant protection products allowed to assign all biostimulants to the category of plant protection products, this conclusion is not satisfactory, as it does not acknowledge the unique features of biostimulants. Biostimulants open up new approaches to the improvement of plant nutrition and of plant protection, yet thet are not fertilisers nor pesticides, and the legal framework should somehow acknowledge this novelty.

With this aim, the following elements could be considered :

1. Biostimulants can be defined by their capacity to modify the plant's physiology, but they group diverse molecules and physiological functions. Many of them aim at improving nutrition efficiency and/or at strengthening plant defenses against stress, but an expanding range of effects may be expected in the future. An option would be to discriminate between these application scopes and to divide biostimulants between different legal categories, based on their main function in plants. Some would be regarded as "fertilisers", others as "plant protection products", still others could form novel categories. For some products, like seaweed extracts, this would not be easy as both nutritional effects and defense elicitation effects are simultaneously described.
2. Fertilisers and plant protection products, in their current EU definitions, include substances of very different chemical natures. Synthetic molecules, including pesticides, herbicide safeners, hormone analogs, are covered by the Plant Protection Products regulation, while relatively simple, inorganic and organic molecules as nutrients providers are covered by the fertiliser regulation. Biostimulants also show contrasting chemical natures, some being synthetic, organic molecules, others being natural, inorganic molecules. Considering that the EU legislation aims at ensuring the safety of the products prior to their market release, the legal framework on biostimulants might be adapted to the existing knowledge on, and "history of safe use" of the molecules intended to be commercialized. This criterion could be instrumental in the definition of the evaluation requirements prior to market release.
3. Both the fertilisers and plant protection products regulations appear to be revisable. However, considering the legal agenda, it appears more likely that the EC Fertilisers Regulation will be revised before any possible amendment of the recently approved Plant Protection Products Regulation. As any legal

text, they need to take into account recent technical advances in their fields. The fertilisers regulation describes nutrients formulations, but did not consider products showing a capacity to improve nutrient use efficiency. The regulation could be expanded to such products. In a similar way, protection of plants against harmful organisms did not intially realize that pesticidal substances would be complemented by plant "immunization" compounds. When hormone analogs and herbicide safeners were introduced under this regulation as compounds "influencing the life processes of plants" (alinea (b) of Article 2), it was apparently not realized that metabolic effectors of another kind would be developed. Legal options need to be identified to acknowledge the specificites of biostimulants, but this task is out of the scope of this scientific review.

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