Plant biostimulants: bioactive composition, mechanisms of action, and physiological responses

María Guadalupe Cárdenas-Barrera [1§](https://orcid.org/0009-0003-4974-385X)
Gloria Azucena Soto-Fernández[1§](https://orcid.org/0009-0002-1632-4133)
Carlos Farfán-Flores[1*] (https://orcid.org/0009-0006-8117-7351)
Silvia Armenta-Jaime[1] (https://orcid.org/0000-0001-8464-5591)
Sergio Hernandez-León[1] (https://orcid.org/0000-0001-5892-6066)
Oscar Arce-Cervantes[1*] (https://orcid.org/0000-0002-3388-2973)
[1]Instituto de Ciencias Agropecuarias, Universidad Autónoma del Estado de Hidalgo, CP. 43775, Ciudad Universitaria, Tulancingo de Bravo, Hidalgo, México.
*Corresponding: *cfarfan170799@gmail.com, oarce@uaeh.edu.mx*
[§] Equal contribution

Abstract
Agriculture faces the challenge of increasing crop productivity under conditions of soil degradation, intensive mineral fertilizer use, and growing pressure associated with climate change. In this context, plant biostimulants (PB) have emerged as a functional alternative to enhance plant physiological performance beyond the direct supply of nutrients. This review synthesizes current knowledge on microbial and non-microbial biostimulants, with emphasis on their composition, mechanisms of action, and effects on plant physiology. Among microbial biostimulants, arbuscular mycorrhizal fungi, *Trichoderma* spp., plant growth promoting rhizobacteria (PGPR), and actinobacteria stand out for their ability to improve nutrient acquisition, modulate hormonal pathways, and enhance stress tolerance through rhizosphere mediated interactions. In contrast, non-microbial biostimulants, including humic substances, protein hydrolysates, seaweed and botanical extracts, chitosan and its derivatives, as well as certain inorganic compounds, act through multifactorial mechanisms associated with nutrient uptake and assimilation, redox regulation, hormonal signaling, osmotic adjustment, and the activation of defense related responses. Overall, the available evidence indicates that plant biostimulants represent a strategic tool to strengthen physiological efficiency, resilience, and sustainability in agricultural systems. However, their rational use still requires a deeper mechanistic understanding, methodological standardization, and validation under real production conditions.

Key Words: Nutrient use efficiency, rhizosphere microbiome, hormonal signaling, redox regulation, stress tolerance

Introduction
Agriculture faces the challenge of increasing crop productivity to meet the growing demand for healthy food under conditions of soil resource degradation and climate change related stresses. To sustain plant growth and yields at the global scale, agricultural production has relied heavily on mineral fertilizers, particularly those based on nitrogen (N), phosphorus (P), and potassium (K) (Bergstrand, 2022). However, the intensified use of these inputs has been associated with nutrient losses, soil degradation, eutrophication of water bodies, and greenhouse gas emissions, thereby compromising the sustainability of agricultural systems (Debasmita et al., 2026).

In this context, plant biostimulants (PBs) have emerged as promising tools to support the transition toward more efficient and resilient production systems (López-Serrano et al., 2026). These products are not defined by their direct nutrient supply, but rather by their capacity to modulate biological and physiological processes in plants and their immediate environment, thereby promoting improved nutrient use efficiency, enhanced tolerance to abiotic stress, better crop quality, and, in some cases, increased nutrient availability in the rhizosphere. PBs include

humic substances, protein hydrolysates, seaweed extracts, plant extracts, silicon, arbuscular mycorrhizal fungi, and plant growth-promoting rhizobacteria belonging to the genera *Azotobacter*, *Azospirillum* and *Rhizobium* spp (Rouphael and Colla, 2020; Roche et al., 2024).
Nevertheless, despite the rapid expansion of both the scientific and commercial sectors, the concept of biostimulation still faces major challenges. One of the main issues lies in the fact that biostimulants comprise an extremely heterogeneous category of substances and/or microorganisms (López-Serrano et al., 2026). This diversity in origin, composition, and formulation has facilitated the rapid development of the field, but has also hindered their classification, the comparison of studies, and the precise identification of the mechanisms underlying their effects (Baltazar et al., 2021; Kaushal et al., 2023). Within this framework, the present review provides an integrative overview of microbial and non-microbial biostimulants, with emphasis on their composition, mechanisms of action, and effects on plant physiology. In addition, conceptual and methodological limitations that still constrain their interpretation are discussed, together with future perspectives for their integration into more sustainable and resilient agricultural systems.

Unlike conventional fertilizers, which are designed to supply essential nutrients directly to plants, PBs act primarily act by modulating biological and physiological processes associated with nutrient acquisition, stress adaptation, and crop performance. Reflecting their growing importance in sustainable agriculture, the global bioinput market has rapidly expanded, with biostimulants representing one of the fastest growing categories of agricultural products (da Silva et al., 2026). According to the European Union Regulation (REPC) 2019/1009, a PBs is defined as a product that stimulates plant nutrition processes independently of its nutrient content, improving nutrient use efficiency, tolerance to abiotic stress, crop quality traits, or nutrient availability in the rhizosphere. Given their diversity in origin and composition, classification based on origin and biological nature provides a useful framework of substances and microorganisms. Therefore, classification based on origin and biological nature provides a useful framework for understanding their modes of action and agricultural applications.

Microbial biostimulants

Microbial biostimulants are formulations based on beneficial microorganisms that enhance nutrient availability and nutrient use efficiency in plants (Chaudhary et al., 2022). Although some of these microorganisms may also function as biocontrol agents, their role in mitigating biotic stress is not considered a primary attribute of biostimulation (Kumari et al., 2023; Fasusi et al., 2023). In this context, microbial biostimulants encompass a wide range of plant associated microorganisms, which can be broadly classified into fungal and bacterial based groups (Fig. 1) (Sani & Yong, 2022).
Fungal biostimulants include species such as *Trichoderma* spp. and arbuscular mycorrhizal fungi (AMF). *Trichoderma* spp. are widely used in agriculture due to their ability to promote plant growth and improve tolerance to abiotic stress through hormonal modulation and the production of bioactive metabolites (Jin and Alberti, 2025). In turn, AMF establish mutualistic symbioses with plants through root colonization and the development of extraradical hyphal networks, thereby increasing soil exploration and facilitating the uptake of poorly mobile nutrients, particularly phosphorus (Begum et al., 2019). In addition, AMF contribute to the maintenance of nutritional homeostasis and improved plant growth.

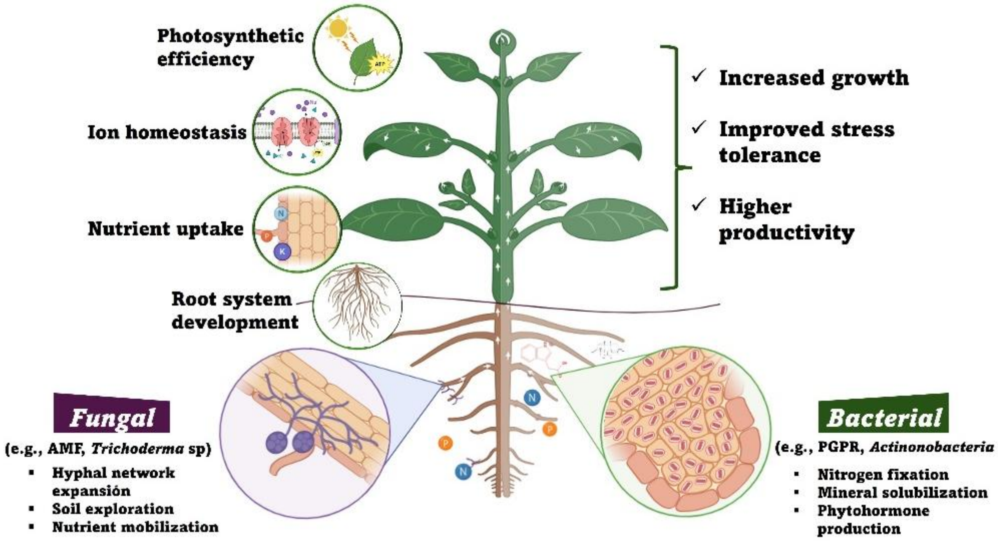

Figure 1. Microbial biostimulants: mechanisms of action and plant physiological response

Bacterial biostimulants mainly include plant growth-promoting rhizobacteria (PGPR) and actinobacteria, particularly members of the genera *Rhizobium*, *Azotobacter*, and *Azospirillum* (Baltazar et al., 2021). These microorganisms produce a wide range of compounds capable of reshaping rhizosphere microbial dynamics and increasing the availability of nitrogen, phosphorus, potassium, and other essential minerals. They are also able to synthesize phytohormones such as auxins, gibberellins, cytokinins, ethylene, and abscisic acid, thereby modulating key physiological processes in plants (Kaushal et al., 2023; Roche et al., 2024). In addition, certain PGPR can modify soil conditions through the production of organic acids, which promote organic matter mineralization and enhance nutrient availability (Chen et al., 2023). Likewise, the production of 1-aminocyclopropane-1-carboxylate (ACC) deaminase enables the regulation of ethylene levels, thereby promoting root elongation and improving tolerance to abiotic stress (Orozco-Mosqueda, et al., 2022; Aloo et al., 2022).

Ecological relationships in the rhizosphere

The concept of biostimulation has evolved, shifting from considering only the direct effects on plants to a more integrative view that recognizes the role of ecological interactions between roots and the microbial communities associated with plant rhizospheres. According to Gen-Jimenez et al. (2025), these ecological relationships represent a second genome or defense system in plants. This perspective considers the plant and its microbiome as a functional unit, called the holobiont, in which the physiological responses largely depend on the complex relationships established among the organisms that constitute the root ecosystem (Rouphael and Colla, 2020; Trivedi et al., 2020; Adedayo and Babalola, 2023).

Plant-soil-microorganism interactions constitute one of the most dynamic and complex biological interfaces, characterized by intense metabolic activity and high microbial diversity. Consequently, the structure of the rhizosphere microbiome is the result of a complex network of ecological interactions modulated by both biotic and abiotic factors (Compant et al., 2021; Trivedi et al., 2023). These mutualistic relationships represent one of the main mechanisms by which biostimulants improve crop growth and productivity under diverse environmental conditions (Santoyo et al., 2021; Kaushal et al., 2023; Ali et al., 2024).

In addition, the ecological interactions of biostimulants promote biological control and competition, as they produce antimicrobial compounds, siderophores, and secondary metabolites that limit the development of competing or potentially pathogenic organisms (Hamid et al., 2021). This competitive exclusion significantly contributes to disease suppression and maintenance of microbiome functional stability (Compant et al., 2021; Adedayo and Babalola, 2023).

Another relevant aspect is the existence of keystone taxa, the activity of which strongly influences the structure and function of microbial communities. Recent ecological network analyses have demonstrated that the presence or absence of specific taxa can significantly alter microbiome stability, consequently affecting plant responses to stress conditions. This knowledge has fostered new strategies aimed at designing biostimulants capable of promoting key microorganisms that contribute to maintaining the ecological functionality of the rhizosphere (Banerjee et al., 2023; Trivedi et al., 2023).

Endophytes and microbial consortia

Endophytic microorganisms are promising biotechnological tools for developing next generation agricultural biostimulants. Their primary mechanisms of action involve the production of phytohormones and plant growth regulating compounds. Several endophytic bacteria belonging to genera such as *Bacillus*, *Pseudomonas*, *Azospirillum*, *Burkholderia*, and *Enterobacter* synthesize indole-3-acetic acid, cytokinins, gibberellins, and other bioactive compounds that stimulate root development, cell elongation and biomass accumulation. Furthermore, many endophytic microorganisms exhibit ACC deaminase activity, which reduces stress induced ethylene concentrations and promotes plant growth under adverse conditions (Orozco-Mosqueda et al., 2021; Ali et al., 2022).

The ability of endophytic microorganisms to increase plant tolerance to drought, salinity, extreme temperatures, and environmental contamination is associated with the induction of antioxidant enzymes, osmoprotectants, and secondary metabolites, which are crucial for maintaining cellular homeostasis and minimizing oxidativa damage. These microorganisms also regulate hormonal pathways involving abscisic acid, jasmonates, and ethylene, thereby strengthening plant adaptation to abiotic stress (Nadeem et al., 2021; Ali et al., 2022; Verma et al., 2023).

Currently, microbial consortia are a promising strategy for agricultural biostimulants because of their ability to promote plant growth through complementary biological mechanisms that enhance productivity and sustainability. These consortia consist of functional communities that integrate plant growth promoting bacteria (PGPB), arbuscular mycorrhizal fungi, actinobacteria, *Trichoderma* species, and other beneficial microorganisms that synergistically interact within the rhizosphere (Santoyo et al., 2021; Ruzzi and Aroca, 2021; Negi et al., 2024).

Recent studies have indicated that microbial consortia act as microbiome-modulating agents, promoting the functional stability of soil microbial communities and improving soil resilience to environmental changes (Compant et al., 2021; Trivedi et al., 2023; Adedayo and Babalola, 2023). Functional complementarity among microorganisms enhances ecological resilience and crop adaptation to adverse environmental conditions, stimulating interest in the rational design of microbial communities for biostimulant applications (Tabacchioni et al., 2021; Negi et al., 2024).

Non-microbial biostimulants

Non-microbial biostimulants are derived from organic and mineral sources and therefore comprise a broad diversity of biologically active compounds (Sagar et al., 2021; Chen et al., 2023). This group exerts its effects primarily through the modulation of key physiological processes, including hormonal signaling, regulation of enzymatic activity, maintenance of redox balance, and improvement of nutrient availability and assimilation at the root level (Du Jardin, 2015; Rouphael & Colla, 2020). In addition, these products have been shown to induce the synthesis of osmoprotectants, modulate the expression of stress related genes, and enhance photosynthetic efficiency (Yakhin et al., 2017; Franzoni et al., 2022). Non-microbial biostimulants are generally classified into five major categories: (i) humic substances, (ii) protein hydrolysates and nitrogen-containing compounds, (iii) seaweed and plant extracts, (iv) chitosan and its derivatives, and (v) inorganic compounds (Kaushal et al., 2023; Roche et al., 2024), as illustrated in Fig. 2. The following sections describe each of these categories in detail, with emphasis on their bioactive composition and the specific mechanisms through which they influence plant physiology.

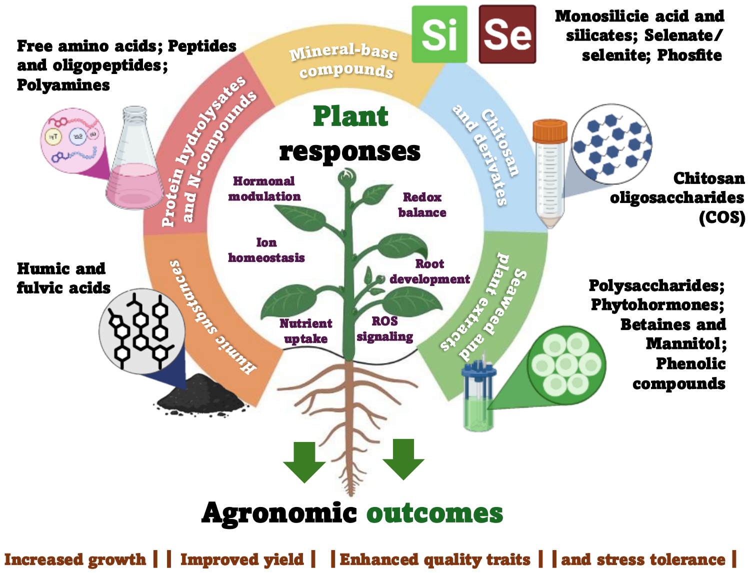

Figure 2. Non-microbial biostimulants: bioactive compounds and plant physiological responses

Humic substances (HS)

Humic substances (HS) are key components of soil organic matter derived from the biological and chemical decomposition of organic residues and play a fundamental role in both soil structure and plant physiology (Maffia et al., 2025). They can be extracted from a range of sources, including organic matter, leonardite, compost, and vermicompost (Lv et al., 2024; Martins et al., 2024). Structurally, HS are commonly classified into three main fractions: humic acids (HA), which are soluble under alkaline conditions and typically range from 20,000 to 100,000 Da in molecular weight; fulvic acids (FA), which are soluble under both acidic and alkaline conditions and have lower molecular weights (approximately 500–2,000 Da); and humins, an insoluble, highly heterogeneous, and recalcitrant fraction in both acidic and alkaline media (Ampong et al., 2022; López-Serrano, et al., 2026).

As a result, most research has focused on HA and FA, whose biostimulant effects are comparatively well documented (Table 1). Among the main reported mechanisms are the enhancement of soil cation exchange capacity (CEC) and the stimulation of proton pump activity through the activation of root plasma membrane H^+-ATPases. These processes promote selective nutrient uptake and, under saline conditions, contribute to reduced sodium (Na^+) accumulation and increased potassium (K^+) uptake, thereby supporting ionic homeostasis and improving crop performance (Alsudays et al., 2024).

Table 1. Mechanisms of action and plant responses associated with humic substances in agricultural crops

Crop	Biostimulant type	Main mechanism	Key plant response	Reference
Oryza sativa (rice)	Fulvic acids (FA)	Upregulation of phosphate transporters (OsPTs); root exudation of organic acids	Improved P acquisition under deficiency conditions	(Lv et al., 2024)
Hordeum vulgare (barley)	Humic substances (HA + FA; K-humate)	Ion homeostasis regulation (Na^+ exclusion, K^+ uptake); improved nutrient use efficiency	Enhanced salinity tolerance and grain yield	(Alsudays et al., 2024)
Triticum aestivum (wheat)	Humic acids (HA)	Hormonal modulation (auxin- and cytokinin-related pathways); root morphogenesis	Increased root hair density and root biomass	(Rathor et al., 2024)
Solanum lycopersicum (tomato)	Humic substances (HA + FA)	Micronutrient chelation and enhanced Fe availability	Increased chlorophyll content and biomass in soilless systems	(Quijia-Pillajo et al., 2024)
Petunia × hybrida	Humic substances (HA + FA)	Enhanced nutrient uptake and metabolic activity under low N	Improved growth and flowering under nutrient limitation	(Martins et al., 2024)
Cassava / Okra	Vermicompost-derived HA	Synergistic interaction with PGPR; enhanced N cycling	Improved yield and nitrogen use efficiency	(Canellas et al., 2022)

In addition, humic substances modulate hormone related responses by upregulating pathways associated with auxin and cytokinin biosynthesis, resulting in changes in root system architecture, including increased lateral root formation and root hair development (Rathor et al., 2024). They have also been shown to induce phosphate transporter expression and stimulate the exudation of organic acids into the rhizosphere, thereby enhancing the mobilization of previously immobilized phosphorus and improving phosphorus fertilizer use efficiency (Lv et al., 2024). Complementarily, HS can increase photosynthetic pigment content and activate antioxidant defenses, promoting osmoprotectant accumulation and mitigating oxidative damage under stress conditions (Canellas et al., 2024; Martins et al., 2024). These effects are also associated with the regulation of key metabolic pathways, including phenylpropanoid metabolism, which is involved in plant defense and adaptive responses (Nabi et al., 2025).

Taken together, these physiological responses enhance the acquisition of macronutrients such as nitrogen (N), phosphorus (P), and potassium (K), even under adverse environmental conditions (Martins et al., 2024). In addition, synergistic interactions between humic substances and beneficial microorganisms have been reported, particularly in processes such as biological nitrogen fixation and phosphorus solubilization, thereby further improving nutrient use efficiency and plant growth (Canellas et al., 2022).

Protein hydrolysates and nitrogen-containing compounds

Amino acids used as biostimulants may be obtained either by chemical synthesis or through protein hydrolysis (Sun et al., 2024). In contrast, protein hydrolysates (PHs) are complex mixtures of polypeptides, oligopeptides, and free amino acids generated by the partial hydrolysis of protein-rich biomass of plant or animal origin (Malécange et al., 2023). Hydrolysis can be achieved through chemical (e.g., NaOH, KOH, HCl), thermal (e.g., superheated water, steam explosion), enzymatic (e.g., proteases, keratinases, cutinases), or combined processes, all of which directly influence the composition and bioactivity of the resulting fractions (Wang et al., 2026). This technological versatility positions PHs as strategic bioinputs for the valorization of agro-industrial residues, including animal derived by products such as feathers, wool, leather waste, and fish residues, as well as plant derived materials such as alfalfa hay and legume seeds (Dhanasekaran et al., 2025; Farfán-Flores et al., 2025).

Beyond their role as a source of organic nitrogen, the biostimulant efficacy of PHs lies in their ability to modulate plant physiological processes (Table 2) (Sun et al., 2024). These compounds can act as signaling molecules, eliciting phytohormone like responses that promote root development and vegetative growth (Malécange et al., 2023). In addition, PHs have been reported to improve nitrogen use efficiency by stimulating key enzymes involved in nitrogen metabolism, particularly glutamine synthetase (GS) and glutamate synthase (GOGAT), thereby enhancing ammonium assimilation and its incorporation into organic compounds such as amino acids and ureides (Engel et al., 2024). This metabolic regulation has been associated with increased ureide and nitrate accumulation, as well as improved crop performance, indicating a more efficient nitrogen metabolism (Engel et al., 2024).

PHs also play an important role in abiotic stress tolerance. Functional amino acids such as proline can act as osmoprotectants, contributing to cellular stabilization and antioxidant system activation, thereby reducing oxidative damage under saline, drought, or thermal stress conditions (Pérez-Aguilar et al., 2023). In parallel, recent evidence suggests that exogenous peptide application may also participate in plant microorganism interactions in both the rhizosphere and phyllosphere, further expanding the functional scope of this category (Segonzac and Moghan, 2019; Chen et al., 2023). However, the bioactive properties of PHs are strongly dependent on the conditions and methodologies used during hydrolysis. Therefore, the development of efficient and reproducible processing protocols remains essential for obtaining consistent and agronomically effective biostimulant products (Mendes et al., 2023).

Table 2. Mechanisms and plant responses associated with protein hydrolysate-based biostimulants in crop systems

Crop	Biostimulant source	Main mechanisms	Key plant response	Reference
Glycine max	Collagen-derived protein hydrolysate	Enhanced N metabolism (GS/GOGAT activity); increased ureide transport	Increased pod number and grain yield	Engel et al., 2024
Capsicum annuum	Animal-derived protein hydrolysates (slaughterhouse residues)	Improved nutrient assimilation and metabolic activity	Increased yield and fruit nutritional quality (vitamin C, minerals)	Ávila-Pozo et al., 2023
Avena sativa	Keratin-derived protein hydrolysates (wool)	Amino acid supply and metabolic stimulation	Increased shoot growth and forage protein content	Farfán-Flores et al., 2025
Lactuca sativa, Brassica rapa	Animal protein hydrolysates (PAPs)	Early-stage metabolic activation; stress mitigation	Improved germination and seedling growth under stress	Pérez-Aguilar et al., 2023
Solanum lycopersicum, Zea mays	Plant- and animal-derived protein hydrolysates	Chelation and improved nutrient uptake; metabolic regulation	Increased biomass accumulation and nutrient use efficiency	Sun et al., 2024

Seaweed and plant extracts

Seaweed and plant extracts constitute a broad category of non-microbial biostimulants characterized by high compositional complexity and functional diversity. Seaweed extracts (SWEs) are primarily derived from macroalgae and are generally classified into three major groups: *Chlorophyceae* (green algae), *Phaeophyceae* (brown algae), and *Rhodophyceae* (red algae). These extracts are obtained through physical processes, such as heat, pressure, or microwave assisted extraction, as well as chemical methods involving solvents, acids, or alkalis, which enable the release of a wide range of bioactive compounds, including polysaccharides, polyphenols, macro and micronutrients, vitamins, fatty acids, and phytohormones such as cytokinins, gibberellins, auxins, abscisic acid, and betaines (Calvo et al., 2014; El Boukhari et al., 2020; López-Serrano et al., 2026).

Because of this compositional complexity, the biostimulant effects of SWEs and plant extracts cannot be attributed to a single dominant bioactive compound. Indeed, studies based on isolated fractions have shown that none of them can fully reproduce the physiological responses induced by the complete extract, supporting the idea that their activity largely depends on synergistic interactions among multiple constituents (Ali et al., 2021). This feature is particularly important when distinguishing this category from protein hydrolysates (PHs), whose biostimulant activity is more directly associated with peptides and amino acids released during hydrolysis, even when derived from plant biomass, often of legume origin (Malécange et al., 2023). In contrast, the activity of seaweed and plant extracts is predominantly linked to the combined action of polysaccharides, growth regulating compounds, fatty acids, sterols, carotenoids, oxylipins, minerals, polyphenols, and other biologically active secondary metabolites (Ali et al., 2021).

At the physiological level, these compounds may act through multiple complementary mechanisms. These include the supply or modulation of hormonal signals involved in cell division, elongation, and differentiation, thereby promoting root and shoot growth; improved water and nutrient uptake through changes in root architecture; activation of defense responses and stress tolerance mediated by elicitor active polysaccharides; enhancement of the photosynthetic apparatus through increased chlorophyll content and metabolic efficiency; and, additionally, improvement of the rhizosphere environment by favoring beneficial soil microorganisms (López-Serrano et al., 2026). Collectively, these mechanisms position seaweed and plant extracts as multifunctional biostimulants with strong potential to improve crop performance, physiological efficiency, and resilience (Table 3).

Table 3. Mechanisms and responses of plants associated with biostimulants based on seaweed extracts

Crop	Biostimulant source	Main mechanism	Key plant response	Reference
Zea mays	Commercial seaweed extract (foliar application)	Enhanced nutrient use efficiency; metabolic and hormonal regulation	Increased grain yield under reduced N and P fertilization	Fatima, 2024
Solanum lycopersicum	*Sargassum* spp. extract	Antioxidant system activation; osmotic stress mitigation	Increased biomass and improved salinity tolerance	Sariñana-Aldaco et al., 2022
Persea americana	*Durvillaea potatorum* / *Ascophyllum nodosum* extract	Root architecture stimulation; hormonal and metabolic modulation	Enhanced root growth, yield, and postharvest fruit quality	Arioli et al., 2024
Solanum lycopersicum	*Moringa oleifera* leaf extract	Hormonal and antioxidant modulation	Increased vegetative growth, yield, and antioxidant enzyme activity	Culver et al., 2012
Lactuca sativa	*Borago officinalis* extract	Metabolic stimulation and nutrient-related effects	Improved growth and crop quality	Bulgari et al., 2017
Zea mays	*Medicago sativa* extract	Nitrogen metabolism modulation (glutamate synthase activity)	Enhanced plant growth	Ertani et al., 2009

Chitosan and its derivatives

Chitosan is the main derivative of chitin, a natural, biodegradable, and biocompatible biopolymer that represents the second most abundant polysaccharide in nature after cellulose (Pichyangkura & Chadcawan, 2015). Its primary sources include the exoskeletons of crustaceans, such as shrimp and crabs, as well as the cell walls of certain fungi (Baviskar et al., 2025; Rojas-Pirela et al., 2024). Chitosan functions as an elicitor capable of activating plant defense responses upon recognition by pattern recognition receptors (PRRs). This interaction triggers signaling cascades involving salicylic acid (SA) and jasmonic acid (JA) dependent pathways, promoting phytoalexin biosynthesis and the establishment of a primed physiological state that enhances plant responsiveness to both biotic and abiotic stresses (Rojas-Pirela et al., 2024; Sun et al., 2023).

In addition to its role in defense signaling, chitosan modulates plant responses to abiotic stress through the regulation of redox and osmotic homeostasis. It has been reported to induce the accumulation of osmoprotective compounds such as proline and γ-aminobutyric acid (GABA), as well as to stimulate the activity of antioxidant enzymes, including superoxide dismutase (SOD), catalase (CAT), peroxidase (POD), and ascorbate peroxidase (APX), thereby contributing to the mitigation of oxidative damage under adverse environmental conditions (Rojas-Pirela et al., 2024).

Chitosan may also influence plant growth through indirect modulation of hormone related pathways, with reported effects on root development and biomass accumulation. Furthermore, due to the presence of amino groups in its structure, chitosan can act as a chelating agent, enhancing nutrient availability while also contributing to the immobilization of potentially toxic heavy metals (Baviskar et al., 2025).

These effects have been supported by studies conducted under both controlled and field conditions (Table 4). In *Solanum lycopersicum*, foliar application of chitosan (300 mg L^{-1}) improved plant growth and yield-related traits, including fruit number and diameter, without negatively affecting fruit quality (Reyes-Pérez et al., 2020). Similarly, in *Abelmoschus esculentus*, chitin-enriched fertilizers promoted greater vegetative development. In medicinal plants such as *Mentha* spp. and *Artemisia* spp., chitosan has also been shown to act as a metabolic modulator, enhancing the synthesis of bioactive compounds (Sun et al., 2023). This provides an integrated overview of the physiological mechanisms induced by chitosan-based biostimulants and their positive effects on plant growth, yield, and stress tolerance in different crops, highlighting their potential as a tool for sustainable agriculture.

Table 4 Mechanisms and plant responses associated with chitosan-based biostimulants in crop systems

Crop	Chitosan-based biostimulant	Main mechanism(s)	Key plant response	Reference
Solanum lycopersicum	Chitosan (300 mg L^{-1})	Defense signaling activation (SA/JA); antioxidant regulation	Increased plant growth, fruit number, and yield without affecting fruit quality	Reyes-Pérez et al., 2020
Abelmoschus esculentus / *Solanum lycopersicum*	Chitin-enriched amendments	Elicitor-mediated growth stimulation; improved nutrient assimilation	Enhanced vegetative growth compared with untreated plants	Rajalakshmi et al., 2025
Mentha spp. / *Artemisia* spp.	Chitosan and nano-formulations	Induction of secondary metabolism; stress-response signaling	Increased phenolic and flavonoid accumulation; improved stress tolerance	Sun et al., 2023
Horticultural crops	Chitosan	Antioxidant activation; improved photosynthetic performance	Increased chlorophyll content and reduced dependency on agrochemical inputs	Baviskar et al., 2025
Multiple crop systems	Chitosan	Hormonal modulation; nutrient chelation; osmotic and redox regulation	Improved plant growth and stress resilience across environmental conditions	Rojas-Pirela et al., 2024

Inorganic compounds

Particularly silicon (Si) and, to a lesser extent, selenium (Se), have gained increasing attention as plant biostimulants due to their capacity to enhance plant growth, physiological performance, and stress tolerance beyond their conventional nutritional role. Although Si is not considered an essential element for most plant species, it is widely recognized as a beneficial or *quasi* essential element because of its contribution to structural reinforcement and stress resilience, especially when supplied as silicates, stabilized silicic acid, or nanoparticle based formulations (Khan, 2025; Kumar et al., 2025; López-Serrano et al., 2026).

One of the main mechanisms underlying the biostimulant effect of Si is biosilicification, whereby absorbed silicon is deposited in plant tissues as amorphous silica, reinforcing cell walls and acting as a physical barrier against pathogens, herbivory, and excessive water loss (Khan, 2025; Kumar et al., 2025). In addition, under abiotic stress conditions, Si contributes to ionic homeostasis by reducing Na^+ accumulation and promoting K^+ uptake, while also enhancing antioxidant defenses and preserving photosynthetic integrity, thereby mitigating oxidative damage (Kumar et al., 2025; Verma et al., 2024). Its efficacy may be further influenced by interactions with mineral nutrition and the rhizosphere microbiome, as co-application with nutrients such as boron or with silicate-solubilizing bacteria has been shown to improve its bioavailability and plant uptake (Da Silva Liber Lopes et al., 2023; Raza et al., 2023).

Similarly, selenium (Se) has been associated with improved stress tolerance through the activation of enzymatic antioxidants, including glutathione peroxidase, catalase, and ascorbate peroxidase, as well as through the accumulation of non-enzymatic metabolites such as phenolics, glutathione, ascorbic acid, vitamins, and photosynthetic pigments (Saed-Moucheshi and Rezaei, 2023). Moreover, Se metabolism is closely linked to sulfur and nitrogen homeostasis, suggesting broader effects on plant ionomics and metabolic acclimation under stress conditions.

Although inorganic elements have not always been strictly categorized as biostimulants, increasing evidence supports the inclusion of Si, Se, and, in some cases, Zn, Cu, and Fe based nanomaterials within the framework of inorganic or nanobiostimulants, provided that they are applied at non-nutritional doses and elicit physiological responses consistent with current functional and regulatory definitions of plant biostimulation (López-Serrano et al., 2026). Nevertheless, their classification remains highly dependent on dose, chemical form, mode of application, and target response, highlighting the need for more standardized criteria to distinguish nutritional from true biostimulant effects.

Edaphoclimatic Factors and Their Influence on Biostimulants

Understanding the effectiveness of biostimulants necessitates examining the interactions among plants, soil, microorganisms, and climate. Soil pH regulates nutrient availability and microbial activity, directly affecting the efficiency of several PBs (Halpern et al., 2015). For example, Xia et al. (2024) demonstrated that

soils with moderately neutral pH conditions favor the beneficial effects of PBs a by promoting greater physiological activity.
The effectiveness of biostimulants is also significantly affected by the content of organic matter. Organic matter content contributes to enhanced water retention, better physical structure, and increased microbial diversity (Heinonsalo et al., 2021). Also, soil texture and structure influence the persistence and mobility of biostimulants. Backer et al. (2022) reported that plant growth promoting bacteria require adequate oxygen conditions to maintain metabolic activity and effectively colonize the rhizosphere.

Regarding climatic factors, Hernández-Figueroa et al., (2022) evaluated biostimulants in green bean crops under water deficit conditions and found significant improvements in growth and water use efficiency. Similarly, Bulgari et al. (2019) reported that seaweed extracts and amino acid-based biostimulants improved the physiological stability of crops subjected to moderate drought stress.
In relation to temperature factors, Colla et al. (2017) reported that certain biostimulants stimulate antioxidant systems and promote the synthesis of protective proteins, thereby reducing the negative effects of heat stress. Similarly, some plant extracts help maintain membrane stability during low temperature episodes.

However, biostimulant effectiveness is highly context dependent, with responses varying according to crop species, environmental conditions, soil properties, and management practices, as recent meta analyses by Li et al. (2022) reported considerable variability in yield responses among field studies, highlighting the importance of genotype, environment, biostimulant interactions when evaluating agronomic performance.

Omics Strategies for Evaluating Biostimulants

The use of molecular biology, metagenomics, and plant physiology tools has enabled a more precise understanding of the mechanisms underlying plant responses to biostimulants. Metagenomics, transcriptomics, proteomics and metabolomics facilitate the identification of genes, proteins, metabolites, and physiological responses associated with the use of PBs applications, providing a system level understanding of cellular processes and enabling accurate characterization of their mechanisms of action (Zuluaga et al., 2023).

Metagenomics is a valuable tool for characterizing shifts in rhizosphere and endophytic microbial communities following biostimulant application. This approach enables the identification of microbial taxa and functional genes associated with nutrient cycling, plant growth promotion, and stress mitigation, thereby improving our understanding of plant microbiome interactions under biostimulation strategies (Baltazar et al., 2021; Kaushal et al., 2023).

To date, transcriptomics is one of the most widely used approaches for evaluating the molecular responses induced by PBs. RNA-Seq and microarray technologies are the main tools that have allowed the analysis of changes in gene expression associated with specific physiological processes. Numerous studies have demonstrated that biostimulants derived from seaweed extracts, humic substances, amino acids, and microorganisms can induce genes involved in energy metabolism, phytohormone synthesis, nutrient uptake, antioxidant mechanisms, and tolerance to drought, salinity, and oxidative stress (Ertani et al., 2013).

Proteomics enables the evaluation of changes in protein abundance and functionality in response to biostimulant application. Mass spectrometry-based techniques, such as LC-MS/MS, allow the identification of proteins involved in photosynthesis, carbon metabolism, cellular respiration, reactive oxygen species detoxification, and defense compound biosynthesis. In addition, proteomics facilitates the analysis of post-translational modifications, such as phosphorylation and glycosylation, which are essential for regulating metabolic pathways and cellular signaling mechanisms (Colla et al., 2014; Zuluaga et al., 2023).

Among omics approaches, metabolomics is one of the most relevant tools for characterizing the complete metabolic effects of biostimulants, as it directly analyzes the metabolites accumulated in plant tissues. This approach provides insights into how biostimulants alter the metabolic pathways involved in antioxidant synthesis, osmotic regulation, energy metabolism, and secondary metabolite production associated with plant defense. Metabolomics also facilitates the identification of metabolic biomarkers linked to favorable physiological responses, contributing to the development of more efficient and targeted biostimulant products (Halpern et al., 2015; Zuluaga et al., 2023).

The integration of metagenomics, transcriptomics, proteomics, and metabolomics has markedly enhanced our comprehension of the intricate biological processes initiated by plant biostimulants. Although each omics approach yields valuable insights at distinct biological levels, their combined application furnishes a comprehensive systems biology perspective. This perspective interconnects microbial dynamics, gene expression, protein regulation, and metabolic responses, thereby facilitating the identification of molecular markers linked to plant growth promotion and stress tolerance. Furthermore, it supports the development of more effective, targeted, and scientifically validated biostimulant formulations.

Future Perspectives and Research Directions

The use of PBs has expanded rapidly in both scientific research and commercial agriculture, creating significant opportunities for more efficient and resilient crop production, while also posing important challenges for their agronomic consolidation. One of the main challenges lies in advancing the understanding of their mechanisms of action at the molecular, physiological, and ecological levels, since many of their effects remain highly dependent on crop species, edaphoclimatic conditions, and agronomic management practices. In this context, it is essential to integrate omics based approaches, including transcriptomics, metabolomics, and proteomics, with field validation trials in order to identify key bioactive compounds, response biomarkers, and the conditions under which their efficacy can be maximized (Mashabela et al., 2025).

In parallel, the integration of PBs with precision agriculture technologies represents one of the most promising avenues for their rational application. The use of remote sensing, real time monitoring, the Internet of Things (IoT), and predictive models could enable biostimulation strategies tailored to genotype, phenological stage, nutrient availability, and environmental conditions, thereby optimizing dose, timing, and formulation while reducing variability in agronomic responses (Khoulati et al., 2025; Mashabela et al., 2025).

Another critical challenge is the need to move toward more harmonized and evidence based regulatory frameworks capable of establishing clear criteria for product characterization, quality, safety, and efficacy. Figure 3 highlights the importance of considering the practical implications for the successful use of biostimulants, including environmental conditions, agronomic management practices, crop specific responses, biostimulant composition and formulation, and economic and regulatory constraints. The primary objective of biostimulants is to enhance plant growth, improve stress tolerance, increase nutrient use efficiency, and improve crop quality. They are not intended to replace fertilizers or pesticides but rather to enhance plant physiological and biochemical processes. Therefore, the effectiveness of PBs application is inherently multifactorial.

The heterogeneity of formulations, together with the lack of standardized methodologies for their evaluation, continues to limit comparability among studies and their large scale adoption. Therefore, strengthening validation schemes under real production conditions and developing more robust and reproducible protocols will be essential to support functional claims and facilitate regulatory acceptance (Khoulati et al., 2025).
Overall, the future of the sector points toward the development of next generation, multifunctional PBs designed to simultaneously modulate nutrient use, plant growth, stress tolerance, and plant microbiome interactions. From this perspective, progress in the field will depend on truly interdisciplinary approaches integrating plant physiology, microbiology, biotechnology, natural product chemistry, soil science, and applied agronomy. If this transition is successfully consolidated, PBs may play a strategic role in the design of more sustainable, resilient, and resource efficient agricultural systems.

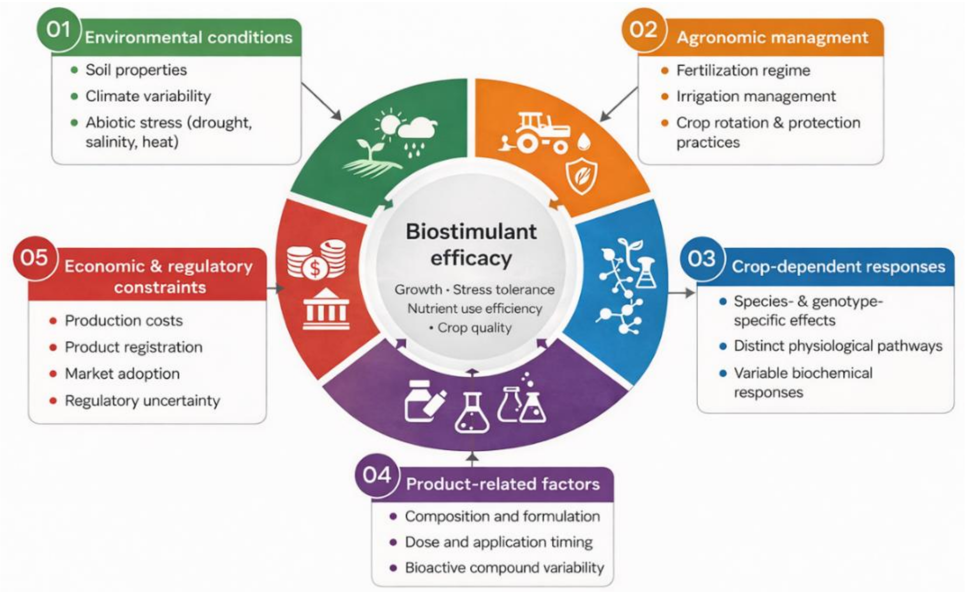

Figure 3. Main factors that determine the performance of plant biostimulants in agricultural systems.

Conclusion

Plant biostimulants have emerged as key components of sustainable agricultural systems because of their capacity to enhance nutrient use efficiency, crop productivity, and resilience to environmental stress. This review highlights that microbial biostimulants including arbuscular mycorrhizal fungi, *Trichoderma* spp., PGPR, endophytes, and microbial consortia primarily enhance plant performance through nutrient acquisition, hormonal modulation, and rhizosphere-mediated interactions. In parallel, non-microbial biostimulants such as humic substances, protein hydrolysates, seaweed and plant extracts, chitosan, and certain inorganic compounds act through multifactorial mechanisms involving nutrient assimilation, redox regulation, osmotic adjustment, and stress signaling pathways.

The evidence analyzed also indicates that the effectiveness of biostimulants is strongly influenced by edaphoclimatic conditions, crop genotype, and management practices, underscoring the need for context-specific validation. Furthermore, the integration of omics approaches, including metagenomic, transcriptomic, proteomic and metabolomic analyses, offers powerful tools to identify bioactive compounds, molecular targets, and physiological biomarkers associated with biostimulant responses. Finally, future progress in the field will require interdisciplinary efforts that combine plant physiology, microbiology, soil science, biotechnology, and precision agriculture. Such integration will be critical for developing next generation biostimulants with improved efficacy, reproducibility, and adaptability, thereby contributing to more sustainable, resilient, and resource efficient agricultural systems.

Author contributions

MGCB & GASF: first authors, investigation, analysis, and writing – original draft & review. CFF: methodology, investigation, data analysis, editing and writing – original draft & review. SAJ: conceptualization and writing – review & editing. SHL: writing – review & editing. OAC: conceptualization, writing – review & editing, and project administration.

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