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# Biostimulants and Their Role in Improving Plant Growth under Abiotic Stresses

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## Abstract

Biostimulants are products that reduce the need for fertilizers and increase plant growth, resistance to water and abiotic stresses. In small concentrations, these substances are efficient, favoring the good performance of the plant's vital processes, and allowing high yields and good quality products. In addition, biostimulants applied to plants enhance nutrition efficiency, abiotic stress tolerance and/or plant quality traits, regardless of its nutrient contents. Several researches have been developed in order to evaluate the biostimulants in improving plant development subjected to stresses, saline environment, and development of seedlings, among others. Furthermore, various raw materials have been used in biostimulant compositions, such as humic acids, hormones, algae extracts, and plant growth-promoting bacteria. In this sense, this chapter aims to approach the use of biostimulants in plant growth according to the raw material used in their compositions as well as their effects on plants subjected to abiotic stresses.

**Keywords:** drought, salinity, temperature, humic substances, seaweed extracts, hormones, arbuscular mycorrhizal fungi, plant growth-promoting rhizobacteria

## 1. Introduction

Biostimulants are natural or synthetic substances that can be applied to seeds, plants, and soil. These substances cause changes in vital and structural processes in order to influence plant growth through improved tolerance to abiotic stresses and increase seed and/or grain yield and quality. In addition, biostimulants reduce the need for fertilizers [1].

Many definitions of biostimulants have been reported [2]. According to [3], biostimulants could be classified depending on the mode of action and the origin of the active ingredient; while Ref. [4] proposed biostimulants should be classified based on their action in the plants or, on the physiological plant responses rather than on their composition. In addition Ref. [1] has emphasized the importance of the final impact on plant productivity which suggests that any definition of biostimulants should focus on the agricultural functions of biostimulants, either on the nature of their constituents or on their modes of actions.

Thus Ref. [2] proposed the following definition of a biostimulant as a formulated product of biological origin that improves plant productivity because of the novel or

emergent properties of the complex of constituents; and not as a sole consequences of the presence of known essential plant nutrients, plant growth regulators, or plant protective compounds. This definition is important as it emphasizes the principle that biological function can be positively modulated through the application of molecules, or mixtures of molecules, for which an explicit mode of action has not been defined.

In small concentrations, these substances are efficient, enhancing nutrition efficiency, abiotic stress tolerance, and/or crop quality traits, regardless of its nutrients content. These substances when applied exogenously have similar actions to the groups of known plant hormones, whose main ones are auxins, gibberellins, and cytokinins [5].

Abiotic stress is a problem of concern for the growth and productivity of plants in modern times. Abiotic stresses, such as drought, salinity, and extreme temperatures, are responsible for huge crop losses globally [6]. In order to prevent these losses, biostimulants are increasingly being integrated into production systems with the goal of modifying physiological processes in plants to optimize productivity [2].

In general, biostimulants are produced as a junction of natural or synthetic substances composed of hormones or precursors of plant hormones. When applied correctly in the crops, it acts directly on the physiological processes providing potential benefits for growth, development, and/or responses to water stress, saline, and toxic elements, such as toxic aluminum [7, 8].

These products, which differ from traditional nitrogen, phosphorus, and potassium fertilizers, may contain in their formula a variety of organic compounds, such as humic acids, seaweed extracts, vitamins, amino acids, ascorbic acid, and other chemicals, which may vary according to its manufacturer [5].

Biostimulants offer a potentially novel approach for the regulation and/or modification of physiological processes in plants to stimulate growth, to mitigate stress-induced limitations, and to increase yield. The effects of biostimulants are still not clear. They can act on plant productivity as a direct response of plants or soils to the biostimulant application or an indirect response of the biostimulant on the soil and plant microbiome with subsequent effects on plant productivity [2].

Several researches have been developed in order to evaluate the use of biostimulants in improving plant growth subjected to abiotic stresses. Furthermore, various raw materials have been used in biostimulant compositions, such as humic acids, hormones, algae extracts, and plant growth-promoting bacteria [7].

In this sense, this chapter aims to approach the use of biostimulants in crops under abiotic stresses and their effects on plant growth.

## **2. Biostimulants and abiotic stresses in plants**

Abiotic stress is defined as environmental conditions that reduce growth and yield below optimum levels [9]. Abiotic stress such as cold, drought, and salt largely influences plant development and crop productivity. Abiotic stress has been becoming a major threat to food security due to the constant changes in climate and deterioration of the environment caused by human activity. To cope with abiotic stress, plants can initiate a number of molecular, cellular, and physiological changes to respond and adapt to such stresses [10].

Abiotic stresses may be prevented by optimizing plant growth conditions and through the provision of water and nutrients and plant growth regulators (PGRs—auxins, cytokinins, gibberellins, strigolactones, and brassinosteroids). In addition to these traditional approaches, biostimulants have been highlighted

as a promoter of optimizing productivity by modifying physiological processes in plants. Biostimulants offer a potentially novel approach for the regulation and/or modification of physiological processes in plants to stimulate growth, to mitigate stress-induced limitations, and to increase yield [2].

The plant hormone auxin is the key regulator of many aspects of plant growth and development, including cell division and stretching, differentiation, tropisms, apical dominance, senescence, abscission, and flowering. The cytokinins are mainly responsible for cell division, besides affecting many other processes, such as vascular development, apical dominance, and nutrient mobilization, especially when interacting with auxins [11].

Gibberellic acid has a marked effect on the seed germination process, activating hydrolytic enzymes, such as  $\alpha$ -amylase and protease, which actively act in the unfolding of the reserve substances, facilitating the mobilization of the endosperm. In addition, they promote the breakdown of dormancy, stem elongation and growth, cell division, and, consequently, leaf expansion [12].

According to Ref. [13], the biostimulant is composed of cytokinin, indolebutyric acid, and gibberellic acid, applied in seed, increased the seedling emergence percentage of *Gossypium hirsutum* L., as well as leaf area, height, and growth of seedlings. The algal extract applied via leaf yielded higher seed yield of *Glycine max* (L.) Merr [14].

An increase in the quantity and quality of *Allium cepa* L. bulbs with foliar application of putrescine and amino acid glutamine was observed [15]. L-glutamic acid is an important amino acid that acts as a central molecule in the metabolism of higher plants [16], being the precursor of the synthesis of chlorophyll in leaves [5], and the carbon regulatory function and nitrogen metabolism [17]. Glutamate is also a precursor of arginine and ornithine, which in turn act on the synthesis of polyamines, which can act on plants, minimizing stress conditions [18, 19]. In addition to these amino acids, others are important in cell metabolism with the expressive diversity of biological functions.

The application of extracts from algae or other plants have beneficial effects on growth and stress adaptation. Algal extracts, protein hydrolysates, humic and fulvic acids, and other compounded mixtures have properties beyond basic nutrition, often enhancing growth and stress tolerance. Although most plant biostimulants are added to the rhizosphere to facilitate uptake of nutrients, many of these also have protective effects against environmental stress such as water deficit, soil salinization, and exposure to sub-optimal growth temperatures [20].

## 2.1 Biostimulants and water stress in plants

Drought is one of the most important and prevalent stress factors for plants in many parts of the world, especially in arid and semiarid areas. Drought stress is a multidimensional stress and generally leads to changes in the physiological, morphological, ecological, biochemical, and molecular traits of plants. In addition, it can negatively affect the quantity and quality of plant growth and yield. Plants respond to water deficit depending on the length and severity of the water deficiency as well as the plant species, age, and developmental stage [21].

Biostimulants when applied to seeds or early plant development stimulate root production and growth [22], especially in soils with low fertility and low water availability, acting on the accelerated recovery of the seedlings in unfavorable conditions, such as water deficit. These products, especially the organic ones, reduce the need of fertilizers to the plants, and increase their productivity and resistance to water and climatic stress, since they act as a hormonal and nutritional increment [23].

Consequently, a series of biostimulants were developed and marketed mainly in the agricultural sector. For example, biostimulants marketed under the trade names Generate, Crop Set, Fulcrum, and Redicrop 2000 worked positively in both the root system and leaf spray in three tree species (*Quercus rubra*, *Betula pendula*, and *Fagus sylvatica*). The biostimulant Yoduo was applied to soybean leaves, reflecting 8.61 bags per hectare more than the control. Stimulate® was applied in sugarcane stalks, resulting in higher productivity and higher profitability index compared with treatment without this biostimulant. Biostimulants CROP + ®, SEED + ®, Carbonsolo ®, Kymon Plus ®, which are composed of arginine, serine, phenylalanine, alanine, aspartic acid, glycine, proline and hydroxyproline, glutamic acid, tryptophan, and valine were used in the isolation and in different combinations, applied via leaf and in soybean treatment. These products caused a greater increase in dry mass and leaf area in soybean plants under water stress [24].

Plants subjected to water stress have their cells damaged by free radicals, but the action of antioxidants, reinforced by biostimulants, is able to decrease the toxicity of these radicals, increasing the defense system of plants, due to the increase in their antioxidant levels. According to Ref. [25], plants with high levels of antioxidants improve root and shoot growth, maintaining a high water content in the leaves and low incidence of diseases, both under ideal conditions of cultivation and under environmental stress.

The water deficit affects several aspects of plant growth, with the most apparent effects of water stress being expressed by the reduction of plant size, leaf area, and crop productivity [26]. In recent years, research and use of products considered as plant biostimulants in plants under water stress have been increasing to obtain higher agricultural productivity. For example, the biostimulant Crop + applied by foliar in tomatoes under water stress provided the highest total soluble (°brix)/titratable acidity index, concluding that the application of this biostimulant increases these indices in tomato fruits, even when under water stress [27]. According to [28], the application of the Seed + ® biostimulant via seed treatments and the Crop + ® biostimulant via foliar application on the total chlorophyll index in soybean under water stress increased the total chlorophyll index in soybean plants, providing greater photosynthetic efficiency of plants.

On the other hand, Ref. [29] evaluated the effect of the amino acid L-glutamic acid, via seed treatment, on the germination and development of *Phaseolus vulgaris* seedlings under water restriction. Thus, different concentrations of the amino acid were applied to the seeds placed on polyethylene glycol hydrated filter paper (PEG 6000) under different osmotic potentials (0, 0.2, -0.4, and -0.6 MPa). Thus, the authors concluded that the concentrations of this amino acid did not favor the development of seedlings, interfering negatively in the germination when the osmotic potential was equal to or lower than -0.2 MPa. In addition, seedling development was drastically affected at the osmotic potential equal to or lower than -0.2 MPa, showing a decrease in germination, root length, and seedling volume.

The effects of kinetin and calcium on the physiological characteristics and productivity of soybean plants subjected to water stress and shading in the flowering phase were evaluated [30], and the application of these products promoted the maintenance of the relative water content and the reduction of leakage of cellular electrolytes. In addition, the application of calcium and kinetin to soybean plants under water deficit and shading did not increase the final grain yield.

Maize (*Zea mays*) is a species sensitive to water deficit and among the management techniques related to the induction of tolerance to water deficit in this plant is the application of biostimulants. Thus [31], tried to characterize the effect of two levels of foliar application of the Carbonsolo® biostimulant on the physiological responses of different maize hybrids with and without water deficit. Thirty days

after sowing, the Carbonsolo® biostimulant, which contains 25% fulvic acids, 50% humic acids, 20% amino acids, and 2% water-soluble nitrogen was applied to the plant. The authors concluded that the foliar application of this biostimulant, in the initial stage of the maize crop, resulted in a higher relative water content in the leaves and a lower difference between leaf temperature and air temperature under water deficit conditions.

An experiment was conducted with Stimulate® biostimulant and different water regimes (full, partial, and non-irrigated irrigation) to evaluate the action of this biostimulant on leaf water potential, relative water content, liquid photosynthesis, transpiration, stomatal conductance, plant height, main root length, total leaf area, and dry shoot and shoot mass of *Eucalyptus urophylla*. Stimulate® reduced leaf water potential and relative water content; however, it promoted increases in transpiration, stomatal conductance, and liquid photosynthesis in these plants [32]. This effect may have helped to promote greater growth, both in plant height and in length of the main root. Stimulate® promoted a deepening of the roots of the non-irrigated plants, is an important response in a water deficiency situation, since it allows the capture of water in deeper layers of the soil, favoring the maintenance of its growth for a longer time. In addition, the Stimulate® biostimulant was used in order to evaluate the application of biostimulants under initial growth and dry tolerance of sugarcane plants under moderate water stress in an experiment. The maintenance of higher rates of photosynthesis, transpiration, and stomatal conductance was observed [33].

According to Ref. [20], the biostimulants for improving plant resilience in water limiting environments should stimulate root versus shoot growth, which would allow plants to explore deeper soil layer during the drought season and stimulate the synthesis of compatible solutes to re-establish favorable water potential gradients and water uptake at diminishing soil water. Similar positive effects can be given by those microbial biostimulants that create absorption surfaces around the root systems and sequester soil water in favor of the plants.

## 2.2 Biostimulants and salt stress in plants

Salt stress is one of the most serious limiting factors for crop growth and production. Salts in the soil water may inhibit plant growth by reducing the ability of the plant to take up water and this leads to reductions in the growth rate. Moreover, if excessive amounts of salt enter the plant in the transpiration stream, there will be an injury to cells in the transpiring leaves and this may cause further reductions in growth. These salinity effects cause ion imbalance or disturbances in ion homeostasis and toxicity; this altered water status leads to initial growth reduction and limitation of plant productivity [34]. The management strategies used for cultivation under salinity conditions may increase the productivity and land use both under and under non-saline conditions. Among these strategies, the application of organic matter and biofertilizers, mycorrhization, foliar application of organic and inorganic substances, and the application of biostimulants are highlighted [35].

Biostimulants based on humic substances have been studied in terms of stress protection against salinity due to their biostimulatory activity [36–38]. For salt-affected soil characteristics, results of [39] showed marked improvements in physical and chemical properties of soil by humic substances and *Moringa oleifera* leaf extract is considered as biostimulants that is used for plant growth under normal and salt stress conditions. The application of humic substance-based biostimulants for plants subjected to saline stress showed a capacity to osmotic adjust by maintaining water absorption and cell turgor [40]. Therefore, these authors consider humic substances-based biostimulants as a vigorous growth biostimulant and a

nutritive means used to protect various crop plants against some environmental stresses, in special, saline stress.

Application of humic acids to common bean (*Phaseolus vulgaris* L.) under high salinity (120 mM NaCl) increased endogenous proline levels and reduced membrane leakage [38], which are both indicators of better adaptation to saline environments. Humic acid extracts applied to rice (*Oryza sativa* L.) played a role in activating anti-oxidative enzymatic function and increased reactive oxygen species (ROS) scavenging enzymes. These enzymes are required to inactivate toxic free oxygen radicals produced in plants under drought and saline stress [41].

The commercial biostimulant Stimulate® presents 0.009% cytokinin, 0.005% gibberellin, and 0.005% auxin, and it has been used in several studies regarding saline stress in plants [42–47]. However, the results are not conclusive about its effect on improving plant resistance under salt stress. On the other hand, the application of the commercial biostimulant Retrosal®, containing calcium, zinc, and specific active ingredients, on lettuce conferred enhanced tolerance when plants were exposed to NaCl treatments, due to its multifaceted action at both biochemical and physiological level. In particular, a significant biostimulant effect was observed on several variables examined, among which fresh yield, dry biomass, chlorophyll content *in vivo*, nitrate concentration, and some leaf gas exchange parameters as well as chlorophyll *a* fluorescence parameters [48].

In addition to these substances mentioned above, biostimulants presenting algae and arbuscular mycorrhizal fungi (AMF), fungi, and bacteria as raw material are bioactive compounds in improving salinity stress tolerance by increasing germination rate, growth characters (length, fresh, and dry weight) of shoots and roots, plant quality, productivity, and yield [2, 20]. Algal extracts target a number of pathways to increase tolerance under stress [21]. Application of algal extracts significantly increased the contents of total chlorophyll and antioxidant phenomenon in wheat plants irrigated with brackish water, exhibiting a strong positive correlation with the increase in fresh weight, grain weight, and yield components [49]. Algal extracts have been used on Kentucky bluegrass (*Poa pratensis* L. cv. Plush) to alleviate salinity stress from saline watering in turfgrass experiments [50].

Many studies have shown that the application of commercial biostimulants based on arbuscular mycorrhizal fungi (AMF) inoculum benefits crops under agricultural saline stress conditions by supporting plant nutrition, influencing plant development (bioregulators), and inducing tolerance to saline stresses (bioprotector) [51]. AMF can contribute to protect tomato plants against salinity by alleviating the salt-induced oxidative stress [52]. According to these authors, this ameliorative effect of mycorrhizal colonization shows significant interactions with cultivar and salt exposure. Enhanced antioxidant enzymes activity and lower lipid peroxidation in mycorrhizal plants may contribute to better maintenance of the ion balance the photochemical reactions in leaves under salinity. Plant growth-promoting rhizobacteria-based biostimulants are considered easy-to-use agroecological tools for stimulating plant growth and enhancing plant nutrient uptake and salt stress tolerance [53]. Salt-tolerant plant growth-promoting rhizobacteria significantly influenced the growth and yield of wheat crops in saline soil [54].

Under salt stress, many authors classified the effects of different categories of biostimulants on plants into direct and indirect influences. The indirect impacts are linked to improvements of physical, chemical, and biological properties of soils, while the direct influences are attributed to improvements of germination, plant growth (root and shoot) as an improvement on resistance of plants to salt stress, as previously mentioned [35].

As one can see, many authors consider biostimulant application as a sustainable tool for plant production and a meaningful approach to counteract salt stress in

plants. In this sense, biostimulant application in agriculture under saline conditions has demonstrated the potential of various categories of biostimulants to improve crop production and to ameliorate salinity stress.

### 2.3 Biostimulants and temperature stress in plants

Temperature stress in plants is classified into three types depending on the stressor, which may be high, chilling, or freezing temperature. Temperature-stressed plants show low germination rates, growth retardation, reduced photosynthesis, and often die. The development of temperature stress can be induced by a high- or low-temperature, and may depend on the duration of the exposure, the rate of temperature changes, and the plant growth stage at which stress exposure occurs. However, plants possess a variety of molecular mechanisms involving proteins, antioxidants, metabolites, regulatory factors, other protectants, and membrane lipids to cope with temperature stress [55].

The temperature factor can be a relevant obstacle to the germination and early development of many horticultural species. Studies have shown deleterious effects on germination when seeds of various crops are exposed to high temperature. Biostimulants are therefore options for mitigating such effects and, by presenting defensive properties against abiotic stresses, such as drought, salinity, and high variation of temperatures; they can alleviate plant defense system of such stressors [1].

Increasing doses of Stimulate® biostimulant (0, 4, 8, and 12 mL L<sup>-1</sup>) as a thermal stress reliever (temperatures 25 and 40°C) on germination and initial growth of melon favored the germination rate by the increase of the doses of biostimulant at both temperatures [56]. Thus, the biostimulant can be used to improve the germination of the melon in high temperature conditions and to improve the initial development of the melon in regions that present high temperatures.

A research was conducted to determine the effects of two biostimulants (humic acid and biozyme) or three different salt (NaCl) concentrations on parsley, leek, celery, tomato, onion, lettuce, basil, radish, and garden cress seed germination at 10, 15, 20, and 25°C. It resulted that two applications of both biostimulants increased seed germination of parsley, celery, and leek at all temperature treatments. In addition, interaction among biostimulants and temperatures was significant in all of the vegetable species [57].

The effectiveness of a product obtained from the enzymatic hydrolysis of porcine hemoglobin (PHH) as a biostimulant that lessen the effect of thermal stress in plants, was observed by two experiments carried out in which lettuce plants were subjected to short-term episodes of intense cold and heat, with different doses of PHH. The results showed that at the highest tested doses, the PHH product ameliorated the negative effects on lettuce growth caused by the increase in temperature and lessened the harmful effects of the cold, i.e., promoted a reaction that lessened the harmful effects caused by the intense cold and heat treatments [58].

In the same way, Ref. [59] evaluating PHH, specifically porcine blood, on strawberry plants in the initial growing stages after being transplanted and subject to conditions of intense cold, an experiment was carried out to compare two doses of PHH with a commercial biostimulant (CB) and a control treatment (C). The results showed that the highest dose of PHH produced more biomass of newly formed roots, that both doses of PHH produced early flowering, and that both doses of PHH led to a significant increase in the early production of fruit compared with the C treatment. None of the biostimulant treatments improved the survival ratio of the strawberry plants compared with the control treatment.

According to Ref. [60], plant thermal acclimation mechanisms include the accumulation of compatible N-rich solutes, such as amino acids, that confer stress tolerance.



Thus, in order to assess the effect of exogenous amino acids treatments, several experiments with plants (lettuce and ryegrass), subjected to three different types of cold stress, were conducted applying an amino acid product obtained by Enzymatic Hydrolysis (Terra-Sorb® Foliar). Results showed that treated lettuce plants have a higher fresh weight than control plants, exhibiting a higher stomatal conductance, which implies productive improvements. In addition, at a high temperature (36°C), ryegrass treated with Terra-Sorb® Foliar showed a superior photosynthetic efficiency (Fv/Fm) and maintains higher levels of chlorophylls and carotenoids. These findings suggest that Terra-Sorb® Foliar has a similar effect to natural plant amino acids and promotes a better more prompt crop recovery from temperature stress.

A major concern in turfgrass management is the summer decline in turf quality and growth of cool-season grass species [61]. Based on this, these researchers investigated whether foliar application of trinexapac-ethyl (TE) and two biostimulants (TurfVigor and CPR) containing seaweed extracts would alleviate the decline in creeping bentgrass (*Agrostis stolonifera* L.) growth during summer months and examined effects of TE and the biostimulants on leaf senescence and root growth. Foliar application of TE resulted in significant improvement in turf quality, density, and chlorophyll content compared with the control. Both TurfVigor and CPR significantly improved visual quality by promoting both shoot and root growth. This study suggests that the proper application of TE and selected biostimulants could be effective to improve the summer performance of creeping bentgrass.

Perennial ryegrass plants treated with a product-based protein and exposed to prolonged high air temperature stress exhibited both an improved photochemical efficiency and membrane thermostability than untreated plants [62]. These results provided consistent and interesting results and showed that foliar applications of protein hydrolysates can positively affect plant tolerance to heat stress [63].

The stress protection of bacterial biostimulants to rainfed field crops can be of particular relevance under increasing temperatures foreseen by most prediction models of climate change. Wheat inoculated with the thermotolerant *Pseudomonas putida* strain AKMP7 significantly increased heat tolerance. Inoculated plants had increased biomass, shoot and root length, and seed size [64].

Bioactive compounds present in the seaweed extracts enhance the performance of plants under abiotic stresses. Spray applications of extracts have been shown to improve plant tolerance to freezing temperature stress. Moreover, commercial *A. nodosum* extract was also reported to promote the performance of lettuce seedling under high temperature stress. In addition, seed germination of lettuce was influenced by priming with *A. nodosum* extract in that germination improved under high temperature conditions [65].

### 3. Final remarks

Biotic stress such as, drought, high soil salinity, heat, and cold is the common adverse environmental conditions that affect and limit crop productivity worldwide.

Plant biostimulants include diverse substances and microorganisms that enhance plant growth and resistance to abiotic stresses and increase seed and/or grain yield and quality. The definition and concept of plant biostimulants are still evolving, which is partly a reflection of the diversity of inputs that can be considered biostimulants.

Agricultural biostimulants may contribute to make agriculture more sustainable and resilient, since a brief review of the literature shows a clear role for a diverse number of biostimulants that have protective effects against abiotic stress.

Biostimulant treatments of agricultural crops have the potential to improve plant resilience to environmental perturbations. In order to fine-tune application rates, biostimulant-plant specificities and techniques are identified that may yield the highest impact on stress protection; high priority should be given to better understanding of the causal/functional mechanism of biostimulants.

Although input-producing companies are investing in the development of new products for the incorporation of biostimulants and additives to agriculture each year, it can be observed from studies carried out that little is known about the mechanisms of action of these inputs in order to optimize the real gains from the incorporation of these products into agricultural production.

In addition, there is a need to address the underlying mechanisms responsible for these effects, given the large number of substances that can be used as biostimulant raw material, such as humic substances, seaweed extracts, plant hormones, and plant growth-promoting rhizobacteria.

The application of an appropriate biostimulant can improve root and shoot vigor, however, the selection of the appropriate biostimulant is critical as the effects can vary markedly between species.

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