www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277-7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2023; 12(1): 1690-1694

www.thepharmajournal.com
Received: 18-10-2022
Accepted: 22-11-2022

Shreya
Research Scholar, Department of Genetics and Plant Breeding, CCS HAU, Hisar, Haryana, India

Arjoo
Research Scholar, Department of Horticulture, MHU, Karnal, Haryana, India

Rajat
Research Scholar, Department of Horticulture, CCS HAU, Hisar, Haryana, India

Corresponding Author:
Shreya
Research Scholar, Department of Genetics and Plant Breeding, CCS HAU, Hisar, Haryana, India

Biostimulants as sustainable stress mitigators: A review

Shreya, Arjoo and Rajat

Abstract
With the advent of climate change, abiotic stresses such as temperature extremities, nutritional imbalance, salinity, drought and flood are not only ravaging cereal crops but are also curtailing the performance of fruit crops around the world. Abiotic stresses have a profound impact on plant growth, development, quality and production and when such stresses occur at the most vulnerable phenological phases of plant growth, the crop yield is reduced drastically. There are several ways to induce abiotic stress tolerance in the target plant species but Biostimulants, both non-microbial and microbial, have been proposed as one of the most sustainable solutions to combat such stresses. They basically stimulate plant growth and development without posing any risk to humans and the natural environment as their chemical counterparts are doing currently. They contain bioactive molecules which along with boosting plant growth, production and quality, improve their ability to cope with environmental adversities. They interact with the primary and secondary metabolism of the plant involved in stress responses and increase antioxidant buildup, allowing for a reduction in plant stress sensitivity. Biostimulants containing anti-stress chemicals, such as proline or glutamic acid, can be used before or during stress conditions, whereas, those involved in the activation of bioactive chemical production, must be used before the stress arises. Biostimulants can be applied as a foliar spray or to the roots, at planting to protect the seedling during the early phases of development, or during flowering or fruit set. Therefore, biostimulants offer immense potential to sustainably mitigate stress-driven adverse impacts on crops.

Keywords: Biostimulants, humic acids, seaweed extracts, chitosan, AMF, PGPR

Introduction
A variety of biotic and abiotic stresses are hostile to the growth and development of plants throughout their lifecycle, ultimately deciding the quality and quantity of plant produce and in the extreme conditions, the survival of plants. Abiotic stress has a more profound impact due to global shift in climate pattern. Abiotic stress is defined as an environmental condition that reduces crop growth and yield below optimum levels, which is currently posing a severe threat to agriculture and the ecosystem, accounting for substantial yield loss and questioning the food security worldwide. Thus, it is imperative to get equipped with multiple sources offering abiotic stress tolerance mechanism in plants to cope with changing climate regimes and meet the demands of the ever-growing population.
Following the pandemic in 2020, a paradigm change occurred worldwide, with consumers beginning to purchase more organic goods as a preventive health strategy. This shift in perspective is anticipated to fuel the organic food market's growth in the future which will further increase the demand for bio-based agro-chemicals in the country. Till date, a plethora of chemical supplements, namely fertilizers, synthetic plant growth regulators (auxins, cytokinins, gibberellins, strigolactones and brassinosteroids), etc. are being used to subdue the losses caused by abiotic stresses but the rising issue of hazards caused by them has turned out to be an alarming signal, obligating to find safe and sustainable alternatives. And biostimulants aptly satisfy the said requirements as they can satisfactorily deal with the predicament without jeopardizing the current ecosystem.

Evolving concept of biostimulants
As the name indicates, Biostimulants include compounds of biological origin that can positively stimulate plant growth and development. They offer a potentially novel approach for the regulation and modification of physiological processes in plants to mitigate stress-induced limitations and providing stress resilience. They are non-nutrient substances that promote plant growth when used in minute quantities (Kauffman *et al.*, 2007) [7]. Another definition delineates biostimulants as "a formulated product of biological origin that improves plant productivity as a consequence of the novel, or emergent properties of the complex of

constituents and not as a sole consequence of the presence of known essential plant nutrients, plant growth regulators or plant protective compounds" (Yakhin *et al.*, 2017) [12]. The notion of plant biostimulants is still evolving, which is partly a reflection of the vast variety of substances implying the potential of inducing stress response in plants and favoring plants' vital processes even during stress conditions. Based on the active ingredient involved, Du Jardin (2015) [1] classified biostimulants under following headings (Fig. 1):

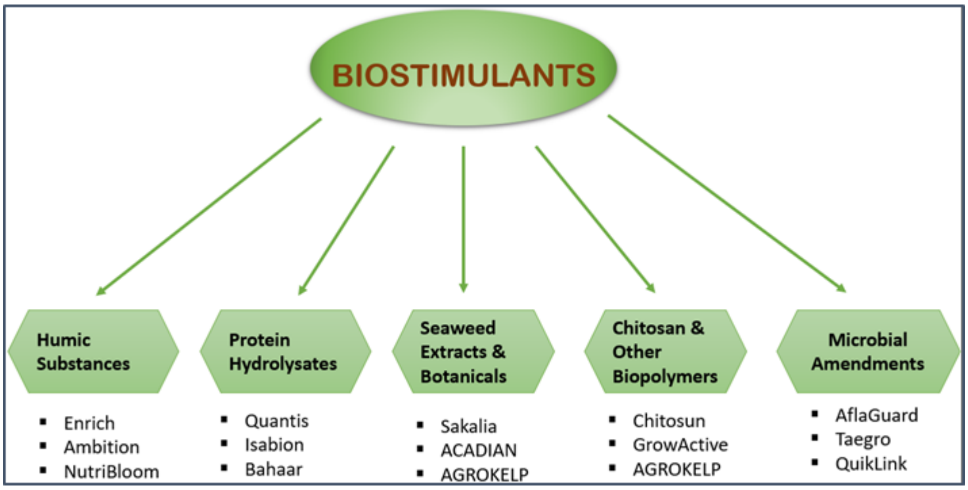

Fig 1: Classification of Biostimulants

A. Humic Acids and Fulvic Acids

Humic and fulvic substances are natural constituents of the soil organic matter, resulting from the decomposition of plant, animal and microbial residues, and also from the metabolic activity of soil microbes using these substrates. Their complexes in the soil result from the interplay between the organic matter, microbes and plant roots. Humic acids are those materials that are insoluble at acidic pH values (pH < 2) but are soluble at higher pH values whereas Fulvic acids are those organic materials that are soluble in water at all pH values. Fulvic acid has twice oxygen content as compared to humic acids which makes it more biologically active. Humic acids increase the permeability of cell walls, making it easier for fulvic acids to carry nutrients into the plant, ameliorating root nutrition. They stimulate plasma membrane H+-ATPases, which convert the free energy released by ATP hydrolysis into a transmembrane electrochemical potential used for the import of nitrate and other nutrients. Both of these work in tandem to optimize growing conditions for plants and can be exploited in forging stress-responsive plants.

Researchers indicated humic acid-mediated hyperactivity of key enzymes involved in the stress response pathway in maize plants. Phenylpropanoid metabolism is essential to the biosynthesis of phenolic compounds, involved in secondary metabolism as well as stress responses. High-molecular mass humic acids have been shown to enhance the activity of key enzymes of this metabolism in hydroponically-grown maize seedlings, suggesting stress response modulation by humic acids (Olivares *et al*., 2015) [8].

B. Protein hydrolysates

These comprise a mixture of Amino-acids and peptides generated by chemical and enzymatic hydrolysis of protein from agroindustrial by-products including plant sources (crop residues) as well as animal wastes (e.g., collagen, epithelial tissues). In addition to modulate N uptake and assimilation, PHs act on signalling pathway of N acquisition by roots, revamping biostimulatory effect. By regulating enzymes of the TCA cycle, they interlink C and N metabolisms. Antioxidant activity is conferred by the scavenging of free radicals by some of the nitrogenous compounds, including glycine betaine and proline, which contribute to the mitigation of environmental stress. Moreover, the beneficial effects of PHs also could be due to the stimulation of plant microbiomes as the substrates provided by PHs, such as amino acids, could provide an ideal food source for these plant-associated microbes.

C. Seaweed extracts and botanicals

As a source of organic matter and as fertilizer, fresh seaweed has been used in agriculture since centuries. However, their biostimulant effects have been reported only recently which led to the commercialization of seaweed extracts and of purified compounds, including polysaccharides, laminarin, alginates and carrageenans, and their breakdown products. In fact, a number of these metabolites are exclusive to their algal source. Most of the algal species utilized for this purpose belong to the phylum of brown algae *i.e., Ascophyllum, Fucus, Laminaria, etc*. Plants sprayed with seaweed extracts exhibit enhanced salt and freezing tolerance. Additionally, they have significant levels of cytokinins, which directly scavenge free radicals brought on by stress and also prevent reactive oxygen species (ROS) formation by inhibiting xanthine oxidation (Fike *et al.*, 2001) [3]. SWEs affect the endogenous balance of plant hormones by modulating the hormonal homeostasis, regulate the transcription of a few relevant transporters to alter nutrient uptake and assimilation.

'Botanicals' describe substances extracted from plants that are used in pharmaceuticals, cosmetic products, food ingredients and plant protection products. A rising number of terrestrial plant extracts with high antioxidant and protective substances have been reported to have biostimulatory effect. Usually, they have complex biochemical composition having different sugars, amino acids, enzymes, hormones, anti-oxidants, flavanols, metal elements etc. in absurd ratios. Some of them were found to have regulatory control over gene expression pertaining to hormonal system, enzyme activation and other physiological stress responses.

D. Chitosan and other biopolymers

Chitosan is a deacetylated form of the biopolymer chitin, produced naturally and industrially. Similar to a plant defense elicitor, this polycationic molecule binds a variety of cellular components, including DNA, plasma membrane and cell wall constituents as well as specific receptors involved in activating defense genes (Katiyar *et al.*, 2015) [6]. Chitosan is found to induce stomatal closure via an ABA-dependent mechanism (Iriti *et al.*, 2009) [5]. It also acts as a chelating agent for heavy metals preventing heavy metal phytotoxicity. Chitosan promotes several defensive genes in plants (e.g., pathogenesis-related genes, such as glucanase and chitinase). In addition, it induces several enzymes in the reactive oxygen species scavenging system (catalase, superoxide dis-mutase and peroxidase). Other similar biopolymers shield the plants by forming a semi-permeable barrier on the surface of the fruit, postponing maturity and senescence, and decreasing the activity of cell-wall degrading enzymes, thereby increasing the shelf-life.

D. Microbial amendments

PGPR (Plant Growth Promoting Rhizobacteria) and AMF (Arbuscular Mycorrhizal Fungi) are the two major microbial classes that are reported to have biostimulant-like properties. Both of these, limit spread of disease-causing microbes via microbial competition. They also prevent pathogen infection by eliciting resistance mechanism such as systemic induced resistance. Some volatile organic compounds (VOCs) related to plant defence under pathogen attack such as geraniol, (E)–2–hexenal, 3–hexenal, benzaldehyde, methyl salicylate etc. are produced as secondary metabolites of the microbial ecology. PGPR population residing in rhizosphere affect the architecture of the root system and interfere with the plant hormonal pathways. Fungal-based products applied to plants that promote nutrition efficiency, tolerance to stress, crop yield and product quality fall under the definition of biostimulants. Major limitation on their use is the technical difficulty to propagate them on a large scale due to their biotrophic character**.**

E. Inorganic compounds

Chemical elements that promote plant growth and may be essential to particular taxa but are not required by all plants are called beneficial elements (Pilon-Smits *et al.*, 2009) [10]. The five main beneficial elements are Aluminium (Al), Cobalt (Co), Sodium (Na), Selenium (Se) and Silica (Si). Their beneficial functions can be constitutive, like the strengthening of cell walls by silica deposits or expressed in defined environmental conditions, like selenium under pathogen attack and sodium under osmotic stress. Other functions include cell wall rigidification, osmoregulation, reduced transpiration by crystal deposits, thermal regulation via radiation reflection, enzyme activity by co-factors, plant nutrition via interactions with other elements during uptake and mobility, antioxidant protection, interactions with symbionts, pathogen and herbivore response, protection against heavy metals toxicity, plant hormone synthesis and signalling.

Mode of action of biostimulants

Biostimulants are mainly used prophylactically with the goal of modifying morpho-physiological processes (Fig. 2) as and when required to curb the negative impact imposed by any kind of environmental stress. Wide variety of biostimulants endorse multifaceted mode of actions briefly summarized as:

- Accelerate root gowth: Biostimulants, when applied to seeds or early stages of plant development, stimulate root production and growth, especially in soils with low fertility and low water availability, resulting in the accelerated recovery of the seedlings in unfavorable conditions such as water deficit.
- Enhance antioxidant buildup: Biostimulants reinforce the action of antioxidants which improve root and shoot growth, help in maintaining a high-water content in the leaves and low incidence of diseases, both under ideal conditions of cultivation and under environmental stress.
- Osmotic adjustment: Application of certain biostimulants under high salinity stress increases endogenous proline levels and reduced membrane leakage, which are both indicators of better adaptation to saline environments. They help in osmotic adjustment by maintaining cell turgor and improving water absorption capacity of plant.
- Ion balance: By providing a better efflux and compartmentation of intracellular ion, enhanced antioxidant enzymes activity and lower lipid peroxidation in plants, biostimulants contribute to better maintenance of the ion balance and photochemical reactions in leaves under salinity stress.

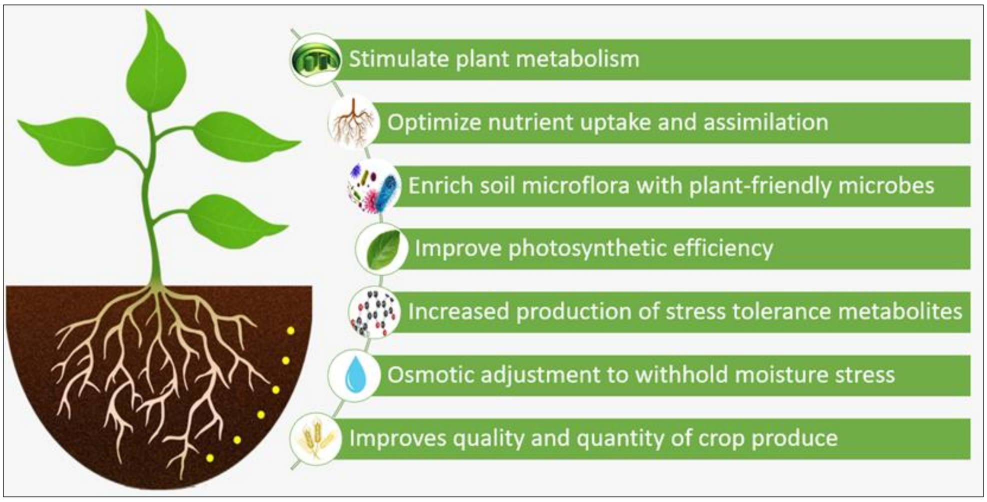

Fig 2: Effects of biostimulants on plant physio-morphological traits

- Chlorophyll content: Plants treated with biostimulants show a superior photosynthetic efficiency, possess membrane thermostability, and maintain higher levels of chlorophylls and carotenoids promoting a prompter crop recovery from temperature stress.
- Phytoalexin production: These are the secondary metabolites of low molecular weight with antimicrobial effects produced in stressed plants. Application of different biostimulants can trigger the synthesis of these phytoalexins through the signal perception for the elicitor signal transduction cascade. This is followed by increase in Ca2+ concentration in the cytosol, production of reactive oxygen species (ROS), a localized hypersensitive response (HR), cell wall reinforcement and stomatal closure.
- Nutrient efficiency: an overall increase in nutrition efficiency can be achieved either through nutrient fixation or direct (HS) or indirect (PGPR and AMF) solubilization of nutrients present in soil in unavailable form.

There are yet other mechanisms namely reduced transpiration by crystal deposits, thermal regulation via radiation reflection, enzyme activity by co-factors, antioxidant protection, interactions with symbionts, pathogen and herbivore response, protection against heavy metals toxicity, plant hormone synthesis and signalling etc. that contribute *inter alia* to the stress resilience among plants.

The nature of the biostimulant is not restrictive in sense that it can be a substance or a microorganism. A substance may be either a single chemical compound or a group of compounds having a well-established biological origin e.g., plant extracts, but not necessarily a fully characterized composition. Due to multifold input categories and their inexplicable mode of actions, commercialization of biostimulants is still facing hurdles. It is yet unclear whether they 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. Consequently, a series of biostimulants were developed and marketed in the agricultural sector though their reach and awareness are yet trivial.

Biostimulants combating abiotic stress

A number of researches have been published till date that unambiguously delineate the role of biostimulants as sustainable agricultural practice for enhancing production and quality of wide array of crops. The application of humic acid in barley under salt stress enhanced growth, yield, concentrations of organic metabolites (syringic acid, alanine, proline, ascorbic acid, glutathione) and phytochelatin 2 (El-Sheshtawy *et al.* 2019) [2]. Improved turf quality, photochemical efficiency and chlorophyll content after application of humic acid in liquid or powder form in creeping bentgrass was reported by Zhang *et al.* (2020) [13]. A similar report was put forward by research of Paul *et al.* (2019) where protein hydrolysate from legume seeds applied to drought-stressed tomato increased biomass, photosynthetic activity and stomatal conductance of plant grown under drought conditions. Licorice root extracts applied to *Phaseolus vulgaris* under salt stress boosted early growth and antioxidant defence, due to their high content of amino acids, gibberellins and selenium. Furthermore, the licorice root extract increased the soluble carbohydrates, which can be used as a source of energy by stressed plants and also causing osmotic modification (Rady *et al.*, 2019) [11]. Biostimulants derived from *Ascophyllum nodosum* have been demonstrated to enhance crop resilience to various stresses. An analysis of the transcriptomes of *Arabidopsis thaliana* plants treated with *an A. nodosum* extract revealed significant increases in polyphenol levels as well as the activation of specific genes linked to plant water scarcity susceptibility (Goni *et al.* 2016) [4].

Future prospects

In order to extend their ambit, high priority should be given to better understanding of the causal/functional mechanism of biostimulants and exploring more plant-based compounds that can work as biostimulants and then experimenting to know their dose, time and rate of application and other specifications. There is a need to fine-tune application rates, biostimulant-plant specificities and techniques that may yield the highest impact on stress protection. The difficulty in identifying modes of action and subsequent standardization of composition of multicomponent biostimulants based on natural raw materials will continue to hamper the use, certification and registration of biostimulants. The solution to this problem will require the collaborative efforts of specialists from different fields. Involvement of stakeholders, farmers, public research and regulatory bodies will be needed to reap the benefits that biostimulants can bring to profitable and sustainable plant productions.

Conclusion

Biostimulants look like a nice-to-have technology instead of a need-to-have technology (as compared with pesticides) and consideration of both immediate and delayed benefits to the farmers, including resource savings and ecosystem services, should be taken care of in the long term. Productive and resource efficient agrosystems would face future needs of food and non-food materials, but they should also deliver ecosystem services which contribute the preservation of soils, water and air. Furthermore, the use of biostimulants can only be successful if the tripartite interactions between the biostimulant, the plant and the environment can be properly addressed. To address these issues, developments in -omics approaches will be critical in accelerating the discovery of mode of action of bioactive compounds. Integrative, multidisciplinary approaches using tools from transcriptomics in conjunction with metabolomics and biochemical analysis are necessary to realize the immense potential biostimulants offer in promoting sustainability with assurance of bounteous yield.

References

1. Du Jardin P. Plant biostimulants: Definition, concept, main categories and regulation. Scientia Horticulturae. 2015;196:3-14.
2. El-Sheshtawy AA, Hager MA, Shawer SS. Effect of bio-fertilizer, Phosphorus source and humic substances on yield, yield components and nutrients uptake by barley plant. Journal of Biological Chemistry and Environmental Sciences. 2019;14:279-300.
3. Fike JH, Allen VG, Schmidt RE, Zhang X, Fontenot JP, Bagley CP, *et al*. Influence of a seaweed extract on

antioxidant activity in tall fescue and in ruminants. Journal of Animal Science. 2001;79(4):1011-1021.

4. Goni O, Fort A, Quille P, McKeown PC, Spillane C, O'Connell S. Comparative transcriptome analysis of two *Ascophyllum nodosum* extract biostimulants: same seaweed but different. Journal of Agricultural and Food Chemistry. 2016;64:2980-2989
5. Iriti M, Picchi V, Rossoni M, Gomarasca S, Ludwig N, Gargano M, *et al*. Chitosan antitranspirant activity is due to abscisic acid-dependent stomatal closure. Environmental and Experimental Botany. 2009;66(3):493-500.
6. Katiyar D, Hemantaranjan A, Singh B. Chitosan as a promising natural compound to enhance potential physiological responses in plant: a review. Indian Journal of Plant Physiology. 2015;20(1):1-9.
7. Kauffman GL, Kneivel DP, Watschke TL. Effects of a biostimulant on the heat tolerance associated with photosynthetic capacity, membrane thermostability and polyphenol production of perennial ryegrass. Crop Science. 2007;47(1):261-267.
8. Olivares FL, Aguiar NO, Rosa RCC, Canellas LP. Substrate biofortification in combination with foliar sprays of plant growth promoting bacteria and humic substances boosts production of organic tomatoes. Scientia Horticulturae. 2015;183:100-108.
9. Paul K, Sorrentino M, Lucini L, Rouphael Y, Cardarelli M, Bonini P, *et al*. A combined phenotypic and metabolomic approach for elucidating the biostimulant action of a plant-derived protein hydrolysate on tomato grown under limited water availability. Frontiers in Plant Science. 2019;10:493.
10. Pilon-Smits EA, Quinn CF, Tapken W, Malagoli M, Schiavon M. Physiological functions of beneficial elements. Current Opinion in Plant Biology. 2009;12(3):267-274.
11. Rady MM, Talaat NB, Abdelhamid MT, Shawky BT, Desoky ESM. Maize (*Zea mays* L.) grains extract mitigates the deleterious effects of salt stress on common bean (*Phaseolus vulgaris* L.) growth and physiology. The Journal of Horticultural Science and Biotechnology. 2019;94:777-789.
12. Yakhin OI, Lubyanov AA, Yakhin IA, Brown PH. Biostimulants in plant science: a global perspective. Frontiers in Plant Science. 2017;7:2049.
13. Zhang X, Goatley M, McCall D, Kosiarski K, Reith F. Humic acids-based biostimulants impact on root viability and hormone metabolism in creeping bentgrass putting greens. International Turfgrass Society Research Journal. 2022;14(1):288-294.