

Review

Biostimulant-Treated Seedlings under Sustainable Agriculture: A Global Perspective Facing Climate Change

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Abstract: The primary objectives of modern agriculture includes the environmental sustainability, low production costs, improved plants' resilience to various biotic and abiotic stresses, and high sowing seed value. Delayed and inconsistent field emergence poses a significant threat in the production of agri-crop, especially during drought and adverse weather conditions. To open new routes of nutrients' acquisition and revolutionizing the adapted solutions, stewardship plans will be needed to address these questions. One approach is the identification of plant based bioactive molecules capable of altering plant metabolism pathways which may enhance plant performance in a brief period of time and in a cost-effective manner. A biostimulant is a plant material, microorganism, or any other organic compound that not only improves the nutritional aspects, vitality, general health but also enhances the seed quality performance. They may be effectively utilized in both horticultural and cereal crops. The biologically active substances in biostimulant biopreparations are protein hydrolysates (PHs), seaweed extracts, fulvic acids, humic acids, nitrogenous compounds, beneficial bacterial, and fungal agents. In this review, the state of the art and future prospects for biostimulant seedlings are reported and discussed. Biostimulants have been gaining interest as they stimulate crop physiology and biochemistry such as the ratio of leaf photosynthetic pigments (carotenoids and chlorophyll), enhanced antioxidant potential, tremendous root growth, improved nutrient use efficiency (NUE), and reduced fertilizers consumption. Thus, all these properties make the biostimulants fit for internal market operations. Furthermore, a special consideration has been given to the application of biostimulants in intensive agricultural systems that minimize the fertilizers' usage without affecting quality and yield along with the limits imposed by European Union (EU) regulations.

Keywords: antioxidant; abiotic stress; biostimulants; climate change; microorganisms; seedling; sustainability

1. Introduction

In the present scenario, the agriculture sector faces concomitant hurdles to increase the crop production to sustain the rising population and maximize resource use efficiency (RUE) while minimizing the environmental effects on the ecosystem and human health [1,2]. Exponential growth in the global human population from 1.7 billion to approximately 7.6 billion in 2019, has resulted in the over-consumption leading to depletion of agricultural systems, such as grasslands being used for pasture, forage, and food production [3–5]. Overexploitation and transformation of grassland into cropland has resulted in decline in the overall agricultural output leading to excessive soil erosion, deteriorated soil structure and decreased soil fertility. Recently, vegetation conservation programs have been implemented to boost biodiversity in agriculture, soil performance, and productivity and also to prevent soil erosion and remove desertification [6]. Several technological advancements have been suggested over the last three decades to maximize the productivity of agricultural production systems via drastically eliminating synthetic agro-chemicals such as fertilizers and hazardous pesticides [7,8]. A reliable, effective and environmentally friendly approach could be the exploitation of biostimulants seedlings and plant-based biostimulants (PBs) (either natural, microbial or organic based), which in synergistic effect may possess the potential to stimulate flowering, fruit setting, crop productivity, plant development, and NUE [9–14]. They act as levers but cannot be used alone especially to protect plants from pests. Zhang and Schmidt, [15] from the Department of Crop and Soil Environmental Sciences, Virginia Polytechnic Institute and State University, described PBs as “substances that, in minute proportions, promote plant growth”. In 2012, EU granted an ad hoc research on PBs to study the role of these preparations which was later published as: “The Science of Plant Biostimulants—A Bibliographic Analysis” [16]. Three years later, a special edition on “Biostimulants in Horticulture” was edited, in which a new concept of biostimulants was suggested, describing the nature, mode of action, and its type of effect on cereal and horticultural crops [17]. In the last decade and officially in compliance with the latest Regulation (EU) 2019/1009, the concept of PBs has been rigorously addressed took into account the following terms: “A PB shall be an EU fertilizer product that stimulates plant nutritional processes [17,18]. It aimed solely towards enhancing one or more of the plant rhizosphere characteristics viz. (i) NUE, (ii) resistance to abiotic stress, (iii) quality or (iv) availability of limited soil nutrients or rhizosphere” [19]. Recently, European Biostimulant Industry Council (EBIC) proposed the agreement globally over the use of PBs. Under the new regulation, “PBs will be CE marked as fertilizing products stimulating plant nutrition processes independently of the products’ after nutrient content. According to Rouphael and Colla [1], the reduced resilience on chemical pesticides, improvement in NUE, and mitigation of negative impacts on environmental factors are the pursuit of agricultural sector.

Under abiotic stress conditions, germination and subsequent cultivation of cover crops are incompetent and sowing is ineffective. Seed enhancement includes seed priming, conditioning, and coating that are often used to boost seed performance during planting, stand homogeneity, seedling growth, and resist pest infestation [20,21]. Seed priming improves germination rate and average seedling under low temperatures, and strengthens wheat stand establishment in marginal soil types [22,23]. The combination of fungicides and fertilizers could enhance the plant stand establishment in perennial ryegrass [24,25]. Seed enhancement via seed coating provide micronutrients to increase the seed germination, seedling growth and stand establishment [26]. According to Traon et al. [27], “A PB is any material or microorganism when applied on plants, seeds or root environments, they stimulate the natural biological processes that benefit the NUE, resilience to abiotic and biotic stresses, regardless of their nutrients composition they contain, or any combination of these compounds/microbes intended for this”. Several terminologies have been documented according to the research findings and observations. Yakhin et al. [13] described biostimulants as “a formulated biologically-originated substance that improves plant productivity as a result of the novel or emerging characteristics of the constituent’s

complex, and not simply as a result of the availability of identified essential plant nutrients, growth regulators and defensive compounds". These are the natural compounds which may have potential applications in plants, seed, and soil. They induce variations in the vital and cellular mechanisms that affect plant growth by improving the abiotic stress tolerance and increase the yield and performance of seed in terms of seed vigor and field emergence. They also decrease the fertilizers' demand [28]. These are the compounds that can improve plant development, but are not labeled as fertilizers, pesticides, or soil alterations [29]. Several definitions of biostimulants have been documented [16,30].

Biostimulants can be applied either as soil- or foliar spray depending on their composition and expected results [31]. These plant based-compounds and other bioactive, natural products like fulvic and humic acids have gained considerable interest over the past two decades [11,12,32,33]. Several biostimulants with their active ingredient have been available commercially (Table 1). After entering into host plant tissues and cells, these materials stimulate biochemical and physiological processes which induce changes in the signaling pathways, synthetic pathways, and hormone regulations involved in growth and development of plants described in Table 1. PBs are produced from the hydrolysis of fruit or vegetable waste, pulses, forages etc. PHs composed of peptides, amino acids, and other non-protein substances. Low-molecular amino acids and polypeptides, phytohormones, enzymes, sugars, antioxidants, and vitamins may provide source of biopreparations.

Table 1. The commercially available biostimulants with functional active components [30].

Biostimulants	Bioactive Components
Algreen [®]	Seaweed extract (<i>Ascophyllum nodosum</i> , <i>Laminaria</i> sp., <i>Sargassum</i> spp.), alginic acid; free amino acids, plant hormones, vitamins
Asahi SL (Atonik) [®]	0.1% sodium 5-nitroguaiacolate, 0.2% sodium ortho-nitrophenolate, and 0.3% sodium para-nitrophenolate
Bio-algeenS-92 [®]	<i>A. nodosum</i> extract
Benefit [®]	Free amino acids, vitamins, and nucleotides
Biozyme [®]	<i>A. nodosum</i> extract, chelated micronutrients, zeatin, GA ₃ , and IAA
Biplantol ^{®®}	Universal macro-and microelements, uronic acids
Bio Rhizotonic [®]	Algae extract, vitamins
Bio Root [®]	Plant-based organic acids, soybean meal, alfalfa, rock phosphate, K-sulfate, and brewer's yeast
Ergonfill [®]	Keratin derivatives, protein hydrolysates, and cysteine
Equisetum extract (Acker-Schachtelhalm extrakt)	Flavonoids, plant acids, glycosides, Si
Fermented plant extract (Fermentierter Pflanzenextrakt) [®]	Lactic acid bacteria, sugarcane molasses, yeasts, photosynthetic bacteria, pepper extracts, grasses, and garlic
Goëmar BM 86 [®]	Algae <i>A. nodosum</i> extract
Grow-plex SP [®]	Liquid humate
Kendal [®]	Glutathione, protein hydrolysate, oligosaccharides, saponins, urea,
KE-Plantasalva [®]	Bio-molasses, herbs extracts
Megafol [®]	Auxin, amino acids, cytokines, gibberellins, betaines, vitamins
Radifarm [®]	Amino acids, betaines, glycosides, microelements, organic acids, polysaccharides, saponins, vitamins
Roots 2	Seaweed, vitamins, humic acid
Root Juice	Fulvic acid, humic acid, seaweed extract
Root & Shoot Builder	Amino acids, natural chelating agents, micronutrients, <i>A. nodosum</i>

Table 1. Cont.

Biostimulants	Bioactive Components
Ruter AA [®]	Micro- and Macronutrients, amino acids,
Slavol [®]	Phosphate-mineralizing bacteria, auxins, nitrogen-fixing bacteria
Tablet [®]	<i>Trichoderma atroviride</i> and <i>Rhizophagus intraradices</i> spores
Terra-Sorb [®]	byproduct of enzymatic hydrolysis of amino acids
Tytanit [®]	Titanium ascorbate
Stimulate [®]	auxin, cytokinin, and gibberellic acid
Retrosal [®]	zinc and calcium
Viva [®]	Polysaccharides, proteins, polypeptides, amino acids, vitamin complexes, and humic acid

Microbial inoculants (beneficial bacterial and fungal agents), biopolymers, seaweed extracts (algal-based), nitrogen derivatives, hormones, humic acids, and herbal extracts are the commonly used biostimulants [19,34,35]. Seaweed-based extracts are the principal source of diverse compounds that may serve as growth supplements, such as antimicrobial compounds, phytohormones, lipids, carbohydrates, proteins, amino acids, and osmoprotectants [36–40]. Among horticultural crops, seaweed extract based biostimulants improves the seed vigor of bean [41] and induced proline biosynthesis in leaves under drought [42]. The definitions of biostimulants often mention microbial inoculants [12], and PHs [11,12,43] that also contribute to increase tolerance against stress and may boost up the nutrient accumulation, mobilization, and their distribution. Biostimulants also help to minimize dormancy, enrich the efficiency of the root system, boost the photosynthetic rate and activities of other vegetative tissues, promotes growth, increase nutrient absorption, enhance crop productivity, seed vigor and consistency, monitor flowering and promote fruit setting, and increase fruit size and ripening [44]. An overview of research on the effect of different kinds of biostimulants on the growth, development, and production of food and ornamental crops has been described in Table 2. All these effects lead to sustainable crop quality, growth, and productivity. Biostimulants and its position in sustainable agriculture is the chief concern of producers, developers, policymakers, scientists, and all those who are interested in them. There is still a question of debate that is how the growth conditions affect the accumulation and biological activity of a biostimulant in a plant.

In recent years, research and applications of biostimulants in agriculture have increased in order to reduce the dependence on less effective conventional pesticides and fertilizers that are typically overused in agricultural crop systems [27,34,45–47]. Seed treatments need even smaller concentration of active ingredients per hectare as compared to the foliar applications predominantly due to reduced surface area and accelerates germination and improves plant growth as compared to non-treated seeds [48]. These substances are effective in small concentrations and promote nutrition, resilience towards environmental stresses, and quality of crops irrespective of their existing nutritional composition [49]. If used exogenously, these compounds may have similar results with defined growth regulating hormones which are primarily cytokinins, auxins, and gibberellins [50]. Utilization of biostimulants as seed coating material has tremendous potential to accelerate the early stand establishment and seedlings' growth. These seedlings are regarded as "biostimulant seedlings" that can be established under harsh and arid conditions and also in soil with poor nutrient management [51]. This review summarizes the diverse applications of biostimulants in intensive agricultural systems that minimize the fertilizers usage with minimal effects on quality and yield. The interpretation of agricultural characteristics (i.e., boost NUE, quality, and resilience towards abiotic stresses) would allow to design a preparation of second generation biostimulant in which synergistic and compatible process may be practically developed and implemented in future studies.

Table 2. Effect of biostimulants on the crop physiology.

Plant Species	Biostimulants	Developmental Stage	Expected Outcomes	References
Tomato (<i>Solanum lycopersicum</i> L.)	Radifarm [®]	Transplants	Enhanced roots growth	[52]
	Radifarm [®] + Megafol [®]	Transplants	Improved nutrients uptake and distribution	[53]
Bell peppers (<i>Capsicum annuum</i> L.)	Radifarm [®] + Megafol [®]	Fruit bearing	Increased macro- and micronutrient, especially Ca ²⁺ ion concentration in leaves and fruits	[54]
	Benefit [®] ; Megafol [®] ; Radifarm [®] ; Viva [®]	From transplanting to harvest	Improved fruit yield	[55]
	Benefit [®] ; Megafol [®] ; Radifarm [®] ; Viva [®]	7th day seedlings	Increased fruit yield	[56]
Garden cress (<i>Lepidium sativum</i> L.)	Acker-Schachtelhalm Extract [®] ; Biplantol Universal [®] ; Fermentierter [®] Pflanzenextrakt [®] ; KE-Plantasalva [®]	Germination	Improved water uptake and seedling growth, germination rate	[57]
Garlic (<i>Allium sativum</i> L.)	Radifarm [®]	After transplanting	Increased seedling after transplanting	[44]
Lettuce (<i>Lactuca sativa</i> L.)	Bio-algeenS-90 [®]	After transplanting	Improved growth and yield characteristics; Increased ascorbic acid content and dry matter and reduced pH	[58]
	Megafol [®] + Viva [®]	Fruit bearing	Activated antioxidative defense mechanism; decreased NK fertilization; enhanced fresh and dry weight	[59]
	Kendal [®] + Megafol [®] + Viva [®]	Flowering and fruit bearing	Increased fruit yield per plant	[60]
Strawberry (<i>Fragaria x ananassa</i> Duch)	Porcine blood-based biostimulant	Before flowering and onset of fruit ripening	Enhanced frost resistance, fruit weight; non-significant effects on fruit yield	[61]
	Radifarm [®]	Transplants	Increased above ground parts and root biomass	[62]
	Radifarm [®]	Robust growth in tissue culture	Enhanced root intensification	[63]
Basil (<i>Ocimum basilicum</i> L.)	Radifarm [®]	After transplanting	Increased seedling growth after transplanting	[44]
	Radifarm [®]	Transplants	Increased nutrient uptake with improved growth	[64]
	Radifarm [®]	After transplanting	Positive effects on morphological traits; enhanced nutrient uptake and proline level	[65]
Marigold (<i>Tagetes erecta</i> L.)	Radifarm [®]	Germination	Enhanced seedling fresh weight and germination energy	[66]
Primrose (<i>Primula acaulis</i> L.)	Radifarm [®]	Transplants	Increased above ground parts and root intensification	[67]
Scarlet sage (<i>Salvia splendens</i> L.)	Radifarm [®]	Transplants	Improved root mass and above ground parts	[64]

2. Source of Biostimulants

Biostimulants are natural raw material formulations. Biostimulants are categorized into microbial and non-microbial biostimulants based upon their source of origin. Microbial source of biostimulants includes consortium of fungi and bacteria, arbuscular mycorrhizal fungi (AMF), fermented products, organic wastes, etc., while non-microbial biostimulants comprised of plant-based products, sea weed extracts, PHs, amino acids, non-protein substances, gelatin mixtures, herbal extracts, humic acid, fulvic acid, etc. Herbal-based extracts, like rosemary promotes the tomato growth [68]. Enzymatic hydrolysis of plants and animals products result in production of diverse range of biostimulants [69]. The hydrolytic products are complex mixture of PHs (amino acids and peptides). The processing of animal based raw materials including, bone meal, casein, skin collagen, and fish waste have been produced by chemical acid or alkaline hydrolysis (Table 3). PBs are produced by the enzymatic hydrolysis for example, fruit or vegetable waste, pulses, alfalfa hay, etc. [70,71]. They stimulate the plants' growth, minimal fertilizers' usage, eco-friendly, and cost-effective [71]. The solution, which simultaneously allows the organic waste to be minimized and biostimulating preparations are produced, is indeed a microbe-based fermentation process. They may also be product of anaerobic digested materials. During fermentation, the dissolved organic matter may also possess the biostimulatory characteristics. The substrates for the dissolved organic matter are generally animal waste, lignin biomass, and plant materials [72]. Low-molecular amino acids and polypeptides, phytohormones, enzymes, sugars, antioxidants, and vitamins may provide marine algae biopreparations. These components trigger the rhizogenesis mechanism and result in favorable anatomical and morphological alterations in plants (Table 3).

Biopreparations from marine algae boost the growth and development in roots of *Cornus alba* (Aurea) by 80% in comparison with the control [73]. The beneficial impacts of seaweed-based extracts as a potent biostimulant have been illustrated by several reports. Among these, *A. nodosum* extracts are documented as the most widely utilized biostimulants [74,75]. Others include, *Ecklonia maxima* [39], *Sargassum johnstonii* [76], *Durvillaea potatumum*, *Sargassum liebmannii*, *Ulva lactuca*, *Caulerpa sertularioides*, *Padina gymnospora*, and *Laminaria* spp., [74,77,78]. The biostimulants' categories also contain consortium of beneficial bacteria or fungi. The most frequently used fungi genus for cultivation of plants are *Heteroconium chaetospora*, *Trichoderma reesei*, *Glomus intraradices*, and *Trichoderma atroviride* (Table 3) [20,79–82]. Rhizobacterial-based biostimulants are incredibly easy-to-use agro-ecological tool which may enhance the nutrients uptake and stimulate plant growth in wheat under salt stress [83]. The plant growth promoting bacterial species include *Enterobacter*, *Ochrobactrum*, *Arthrobacter*, *Pseudomonas*, *Rhodococcus*, *Bacillus*, and *Acinetobacter* [84,85], while the beneficial rhizobacterial species groups includes *Rhizobium*, *Bacillus*, *Pseudomonas*, and *Streptomyces* which effectively acts as biocontrol agents [20]. *Streptomyces* spp. showed substantial protective role against *Pectobacterium carotovorum* subsp. *brasiliensis*, a putrefactive bacteria [86]. The endophytic bacteria isolated from root nodules of soybean elicits protective mechanism against fungal pathogen *Phytophthora sojae* [87].

Table 3. Effect of biostimulants on plant metabolic and physiological responses.

Source	Bioformulations	Plant Response
Consortium of beneficial fungi	<i>Heteroconium chaetospora</i> , <i>Glomus viscosum</i> , <i>Glomus claroideum</i> , <i>Rhymbocarpus aggregatus</i> , <i>Glomus etunicatum</i> , <i>Trichoderma</i> spp., <i>Rhizophagus intraradices</i>	The beneficial fungi promotes the growth and yield in tomato fruit [88] Stimulates protection against oxidative stress [89]
Marine algal biopreparations	<i>Gelidium pectinatum</i> , <i>Sargassum wightii</i> , <i>Enteromorpha intestinalis</i> , <i>A. nodosum</i> , <i>Ecklonia maxima</i>	Enhanced antioxidant capacity, chelation, extended shelf life of fruits, thermal and drought resistance [39,88]
Hydrolytic products	alfalfa hay, fruit and vegetable waste, pulses; natural and chemical (feathers, skin collagen animal tissue, casein, bone meal, fish waste)	Improved yield [90], Enhanced NPK content and macro- and micronutrients in leaves [91,92] High protein content in cereals [91] Biotic and abiotic stresses tolerance [93] Improved soil fertility [94]
Anaerobic digested products	Lignin biomass, plants, and animal	Auxin-like properties [71,95] Improved nutrient availability [96]

3. Biostimulant Applications for Crop Agronomy

The role of bioactive compounds in signaling of primary and secondary metabolic pathways has been generally correlated with promoting germination, plant growth and crop productivity under the influence of PBs [97]. Hydrolyzed collagen of various forms, such as gelatin mixtures of hydrolysates and amino acids, and granulated gelatin inducing the formulation of gelatine was evaluated for cucumber growth [47]. Gelatin hydrolysates regulated the expression of permeases encoding genes (*AAP₃* and *AAP₆*) and nitrogen and amino acids transporters. Hence, the authors inferred that gelatin hydrolysate may be used as a reliable nitrogen (N) source. Moreover, Luziatelli et al. [95] performed a greenhouse experiment on lettuce to assess the impact on three commercially available PBs: vegetal-derived protein hydrolysate (PH), vegetal-derived tropical plant extract, and Cu supplemented PH and epiphytic bacterial colony. Results showed that PBs enhanced the fresh weight of shoot with no significant variations among the organic PBs. They also showed that PBs may boost epiphytic bacterial growth (*Acinetobacter*, *Bacillus*, and *Pseudomonas*) with the aid of PGP and biological pathogens control activity, thus working in synergy with PB organic compounds to improve the fresh lettuce production for marketing. Moreover, Mahnert et al. [96] demonstrated the biostimulant ability comprising of stone dust, malt sprouts, and organic herbs to assess the beneficial effect on growth, development, and efficiency by the field application of microbiota and also in the surrounding. Furthermore, Lucini et al. [98] conducted study to evaluate the metabolomics and physiological responses in melon via the application of lateral root promoting peptides, lingo-sulphonates and micronutrients, a combination of biopolymer-based biostimulants. Different doses (0, 0.3, 0.6, 1.2, or 2.4 L ha⁻¹) of vegetal-based biostimulants were applied around the collar tissue. The substrate drench elicits dose-dependent biomass accumulation in melon transplants. The root characteristics in biopolymer-treated plants were significantly higher at 0.24 mL as compared to 0.06 mL per plant. The enhanced accumulation of shoot and root biomass might be due to involvement of direct and indirect physiological processes in biopolymer-treated melon transplants. For example, signaling molecules specifically, the bioactive peptides and lignosulfonates may trigger signal transduction pathways by induction of target endogenous phytohormones [99]. Palumbo et al. [100] observed that humic acid extracted from municipal solid waste and olive mill water filters might be utilized as reliable biostimulants in dose-dependent manner in maize to enhance the plant growth, marker enzyme activities and nutrient mobilization. Ertani et al. [99] studied the effect of

seaweed-based extracts, viz., from *Laminaria* and *A. nodosum* as potential biostimulants with a concentration of 0.5 mL L^{-1} in maize. By implication of different biochemical and morphological approaches, *A. nodosum* extract significantly promotes the characteristics of root morphological because of higher levels of indole-3-acetic acid (IAA). These results demonstrate the effectiveness of stability characteristics of commercially available algae extracts predicting the cellular targets prior to commercialization (Figure 1).

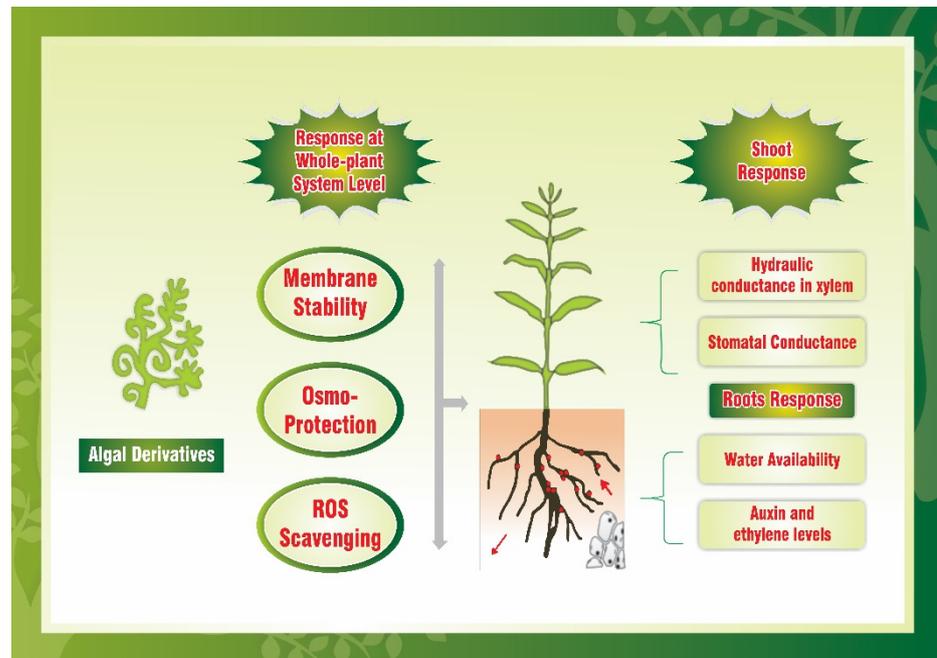


Figure 1. The key metabolic processes at the whole-plant level and physiological level are targeted by algal-based biostimulants [101].

In addition, the major plant growth traits, grain yield and its components were seen in two pepper varieties where *Cladosporium sphaerospermum* was applied to the seedlings [102]. Similar results were observed in tobacco exposed to *C. sphaerospermum* with significantly maximum plant growth. This may be due to induction of putative biochemical and molecular pathways like, photosynthesis, phytohormone homeostasis, cell expansion, and defense responses. In regard to ornamental crops, animal-based PH significantly affected the agronomical and morpho-physiological behavior of snapdragon hybrids as foliar pulp or substratum drench in three concentrations (0, 0.1 and 0.2 g L^{-1}) [103]. At both PB levels, animal PH treated plants particularly at the substratum drench, boosted quality traits and the ornamental characteristics of plants in cultivar-dependent manner in comparison to the control.

In contrast to microbial and non-microbial stimulation activity, PBs may have dual effects involving resistance to diverse range of stresses by using certain natural substances or microorganisms. Sharma et al. [104] reported that exogenous use of jasmonic acid may enable the mustard (*Brassica juncea*) seedlings to be recovered from adverse effects of oxidative damage induced by pesticides across the enhanced expression of *P450*, *RUBISCO*, *CXE*, and *NADH* by inducing plant's antioxidant defense mechanism. Likewise, after challenged environments of *Fusarium oxysporum* f. sp. *lycopersici*, bio-priming of *Trichoderma erinaceum* stimulated the defense transcriptome in tomato, where the following adaptations: (i) enhanced accumulation of defensive proteins, like *WRKY* (a category of proteins bound with DNA) [105], (ii) enhanced antioxidant defense mechanism, and (iii.) increased accumulation of lignification of the cells resulting in increased plant growth [106,107]. At last, Dal Cortivo et al. [108] demonstrated that the antagonist of fungicide action, i.e., sedaxane, a succinate dehydrogenase enzyme showed a significant hormonal activity in

maize seeds which were related to auxin and gibberellin. The authors suggested that the application of sedaxane may promote root production and enhance the N accumulation and phenylpropanoid metabolism in young maize seedlings, thereby eliminating abiotic stress constraints at early development stages. Thus, PBs has tremendous potential to mitigate the toxic effects of synthetic pesticides via. Enhanced expression of genes governing tolerance to oxidative damage.

4. Implications of Biostimulant for Abiotic Stress Tolerance

Abiotic stress is described as environment conditions such as salinity, cold drought, and heat which reduces the crop development and yield below the optimal levels worldwide [6,109]. Owing to the rapid changes in temperature and the deteriorating climatic changes, abiotic stress is becoming a significant threat to sustainable agriculture and food security imposed by human activity [110–114]. Under stressed conditions, plants may elicit a wide range of biochemical, physiological, and molecular alterations to respond and acclimatize under these changing conditions [115]. The effect of biostimulants under diverse range of environmental stresses are depicted in Figure 2. The strategies adopted by the plants includes optimization of plant growth, accumulation of water and mineral nutrients and increased synthesis of plant growth regulators, such as cytokinins, auxins, gibberellins, brassinosteroids, and strigolactones. Biostimulants should be applied as they are growth regulators which have a marked influence on the regulation of physiological processes, seed germination, increase yield, reduce senescence, abscission, promotion of nutrient mobilization, breakdown of dormancy, and increased the seedling emergence [116–118].

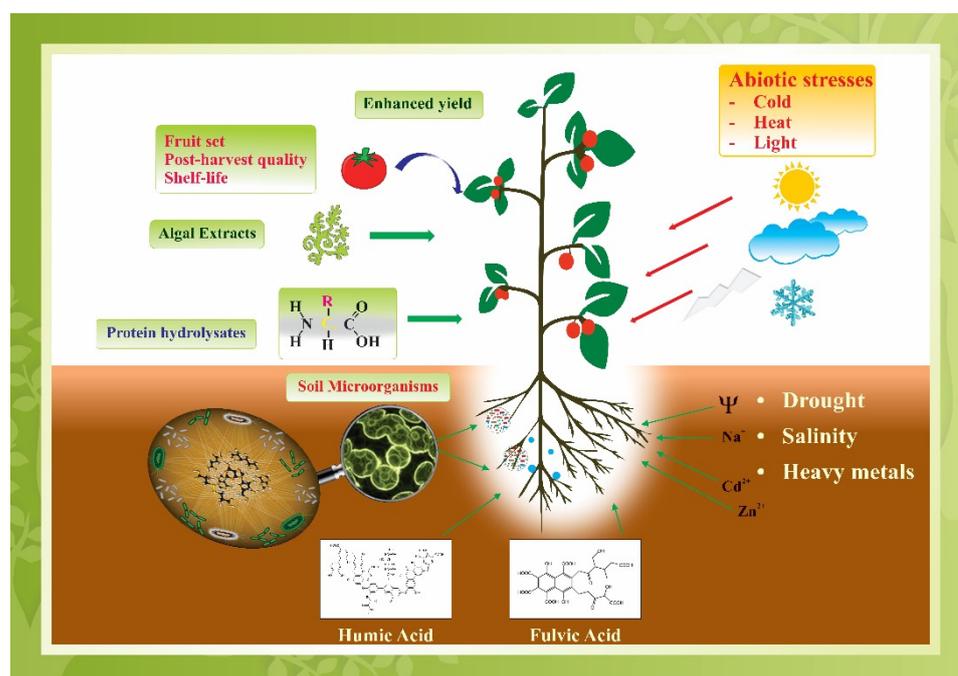


Figure 2. Biostimulants induced physiological effects and tolerance to abiotic stresses [101].

4.1. Drought

Drought is one of the major widespread abiotic stresses especially in the arid and semi-arid zones. It is a multi-dimensional stress which may leads to alterations in the morpho-physiological, molecular, biochemical, and ecological characteristics of the plants [2,6,114]. It adversely affects the quality and quantity of growth and development in plants. The plants adapted to drought conditions primarily based on the duration and severity of water deficiency as well as age, growth stage, and plant species [119]. Biostimulants promote root biomass growth particularly in soils that have low fertility rates and limited water availability. Under drought, direct application to the seeds or at an initial stages of development

accelerated the seedling recovery and induce growth [120]. The effect of L-glutamic acid as a biostimulants was studied on the *Phaseolus vulgaris* seedlings at early growth stages under drought. Different doses of L-glutamic acid on polyethylene glycol (PEG 6000) hydrated filter papers did not significantly improve the germination rate of *P. vulgaris* seedlings. The physiological indices, biomass (fresh and dry matter), and seed vigor were not increased at desired rate after the application of L-glutamic acid, thus may not considered as effective biostimulants [121]. The application of kinetin and calcium as biostimulants significantly maintained relative water content (RWC) and reduced the cellular electrolytes leakage in soybean [122]. The induction of drought tolerance in maize (*Zea mays*), a drought susceptible crop, is one of the effective management strategies which can be achieved by the application of biostimulants. The foliar application of the Carbonsolo[®] biostimulants (50% humic acids, 25% fulvic acids, 2% water-soluble nitrogen, and 20% amino acids) lead to an improvement in RWC in leaves and low temperature differences between the inner leaf environment and surrounding temperature [123]. Biostimulants have a strong impact on physiological indices of the plants. The use of Stimulate[®] biostimulant in *Eucalyptus urophylla* reduced the RWC and leaf water potential; though, it stimulated an increases in photosynthesis, stomatal conductance, and transpiration. Stimulate[®] improved the deepening of roots in non-irrigated plants, a vital response in water deficit situations; it also helps water to be retained in the innermost layers of soil and encourages the survival of plant development for a long period of time [101]. Stimulate[®] also promoted transpiration, stomatal conductance, and higher photosynthetic rates in sugarcane [124]. Under water limiting conditions, biostimulants improve plant resilience by stimulating root growth in spite of shoot growth, allowing the plants to reconnoiter the lower layers of soil during dry season and promote the compatible solutes (proline and glycine betain) biosynthesis to restore the desirable water potential gradients and its intake in soil with less water availability [125].

4.2. Salinity

Soil salinity is among the most critical barriers to the growth and production of crops. Saline water may hinder plant development by lowering the plants' ability to absorb water which can lead to decline in growth rate. In addition, if excessive salts enters into the transpiration stream of plants the cells and tissues in transpiring leaves will experience damage which may results in further reduction in plant development [2,6,109]. The effects of salinity induces ion disruptions of ion homeostasis, alters water status, increase reactive oxygen species (ROS) toxicity which may contributes to preliminary growth reduction and restraints in crop development [126]. The efficient management strategies utilized for cultivation in saline soils includes the application of biostimulants in the form of mycorrhizal growth (AMF), foliar spray of organic and inorganic materials, and the organic matter along with biofertilizers.

Humic acid-based biostimulants had been reported against protective role under salinity [127,128]. Humic acid biostimulants not only improved the soil texture but also improves its physical and chemical characteristics [129,130]. They also have the capacity to adjust osmotic potential by maintaining cell turgor and water absorption under saline conditions [131]. Hence, it is considered as a vigorous growth stimulating biostimulant which protects different crop species against several environmental conditions, especially, under salt stress [130]. Foliar application of humic acids increased the endogenous synthesis of proline and decreased the membrane leakage in common bean (*Phaseolus vulgaris* L.) [128]. It also plays a protective role by activating antioxidative defense mechanism and reduced the release of ROS. These enzymes are involved to detoxify the free radicals of oxygen generated under drought and saline conditions [132,133]. The commercially available biostimulants Stimulate[®] comprised of 0.005% auxin, 0.009% cytokinin, and 0.005% gibberellic acid has been successfully utilized in mitigation of salt stress in plants [125,134–137]. Another commercialized biostimulants Retrosal[®], composed of zinc, calcium, and an active ingredients also conferred enhanced tolerance in lettuce due its multifaceted response

both at biochemical and physiological level [136]. Therefore, biostimulants have a strong correlation with physiological mechanism of plants which may directly influence the photosynthetic rate, biomass accumulation, dry biomass, nitrate concentration, chlorophyll content in vivo, chlorophyll a fluorescence as well as leaf gas exchange characteristics.

Biostimulants with bacteria, algae, and AMF as a raw material represent the bioactive molecules for enhancing the resistance to salt stress by raising the rate of germination, growth characteristics of the roots and shoots (fresh and dry weight, and length), the quality, productivity, and crop yield [20,30]. Algal extracts have a significant effect on plants defense mechanism and target several pathways with the aim of increasing stress resistance [135]. It stimulates the photosynthetic machinery to enhance the chlorophyll synthesis, induce antioxidative phenomenon and exhibited a positive correlation with fresh weight and grain yield in wheat [135]. Algal extracts have been used to mitigate the toxic effects of salts in Kentucky bluegrass (*Poa pratensis* L.) [136]. Symbiotic arbuscular mycorrhizal fungi (AMF) has a strong impact on cultivation efficiency of crops. AMF along with *Rhizophagus irregularis* enhanced the growth potential of *Stevia rebaudiana* [82]. The administration of commercially available biostimulants dependent on AMF inoculum promotes the nutritional status, affects growth and contributes to salt stress resistance (bioprotector) in agricultural and salt-prone areas [138,139]. In tomato, AMF induced antioxidative stress markers to alleviate the toxic effects of salinity [140]. The ameliorative effect of AMF has positive interactions with cultivar type and duration of salt exposure. Increased production of antioxidative enzymes and lower levels of membrane peroxidation in AMF treated plants would further lead to maintain ion homeostasis and photosynthetic reactions in leaves [141]. Therefore, different categories of biostimulants have direct and indirect effect on plant physiology. The direct effects include increased seed germination, seedlings root and shoot growth, and improved resistance to salt stress while indirect impacts includes physico-chemical, and biological characteristics of soils [20,51,127,130]. Henceforth, application of microbial biostimulants is regarded as a key perspective to counteract the effects of salinity and improve the crop production.

4.3. Thermal Stress

Temperature stress in plants is defined according to three forms: heat, cold, and freezing. Temperature-stressed plants exhibit poor germination rate, retarded growth and reduce photosynthetic efficiency, and often fatal [115,140]. Temperature stress can develop at high or low temperatures, exposure duration, temporary fluctuations in temperature and the developmental stage at which stress is imposed [142]. Several reports indicated the deleterious effect of temperatures on several crop species which ultimately reduced the seed germination rate and thereby, decreased the yield potential. Biostimulants offer a sustainable approach for alleviation by defensive properties against stress conditions and boost the plants' defense system [20,130]. Biostimulants act as thermal stress reliever in plants. With increasing doses of Stimulate[®] (4, 8, and 12 mL L⁻¹) the initial growth and germination rate was increased in melon seedlings [141]. The combined effect of humic acid and biozyme applied as biostimulants on tomato, garden cress, parsley, basil, radish, celery, onion, and lettuce at different range of temperatures increased the germination percentage in comparison to control. The synergistic effect reduced the negative impact of heat during seedling germination and boost the defense mechanism while in control plants, germination reduced significantly [143,144].

Porcine hemoglobin (PHH), a hydrolytic product reduced the effect of thermal stress in lettuce when the plants have been exposed to short durations of heat and cold along with different concentrations of PHH. The authors indicated that PHH ameliorated the negative effects of heat and cold in lettuce. The short-term exposure of intense heat and cold were imposed on lettuce plant. Administration of different doses of PHH promoted a reaction that lessened the harmful effects of cold and heat. The physiological parameter, viz., specific leaf weight, fresh and dry matter, relative growth rate were enhanced in dose-dependent manner [145]. Similar results were observed in strawberry when PHH,

specifically was applied during the initial growth stages. The results showed porcine blood led to significant accumulation of biomass at early flowering and early production of fruit as compared with control plants [146]. Exogenous application of Terra-Sorb[®] Foliar, a byproduct of enzymatic hydrolysis of amino acids promoted tremendous increase in fresh weight, stomatal conductance, and higher levels of chlorophylls, carotenoids and photosynthetic efficiency (Fv/Fm) at 36 °C in ryegrass. Thus, Terra-Sorb[®] Foliar showed comparable effects to amino acids and improved the crop recovery from thermal stress [147]. Similarly, trinexapac-ethyl (TE) in creeping grass (*Agrostis stolonifera* L.) [146], product-based protein in turfgrass [148], *Pseudomonas putida* strain AKMP7 inoculum in wheat [149,150], *A. nodosum* extract in lettuce promoted shoot and root growth, chlorophyll content, retard leaf senescence, increase membrane thermostability, seed size, biomass, and resistance against thermal stress [39,151].

5. Implication of Biostimulants on Antioxidant Potential

Biostimulants have a vital effect on metabolic and physiological processes in plants such as the protection of photosynthetic machinery against photo-damage, generation of reactive oxygen species, elevated level of antioxidants, and increased synthesis of ion transporters. The biostimulatory effects regulate several metabolic and cellular processes which consequently benefits socio-economic and environmental aspects (Table 4) [152–154]. A widely observed fruit and vegetable product is antioxidant function. Compounds which prevent the growth of tumor cells and protect them from oxidative stress induced by excessive free radicals are known as antioxidants. Oxidative stress results in DNA damage, antioxidative enzymes, and cell membrane integrity [155]. Biostimulants have a direct effect on antioxidative defense mechanism especially on phenolic compounds, lycopene, and ascorbic acid. Reactive oxygen species (ROS), e.g., O²⁻, OH, and hydrogen peroxide (H₂O₂), are scavenged by antioxidant molecules (antioxidants, viz., phenols, ascorbic acid) and antioxidative enzymes (e.g., catalase (CAT), peroxidase (POX), and superoxide dismutase (SOD) etc. [156]. Applied as a tomato biostimulant, protein hydrolysate had no effects on polyphenolic compounds although their influence on ascorbate and lycopene was recorded. After utilizing an appropriate dose, lycopene content enhanced progressively by 18.0 (2.5 mL/L) and 34.9% (5.0 mL/L) in comparison with control. The 2.5 mL/L dose enhanced the concentration of ascorbate by 27.3% [157]. Biostimulants used in apricot trees boosted the level of antioxidant capacity. During the first season, the fruit's antioxidant potential (76.8 mg/100 g) was higher following the use of the stimulants than in the second season (66.5 mg/100 g). Variations in the climate condition have been found to justify the differences in antioxidant abilities between the two seasons [158].

Salicylic acid (SA) and chitosan-based nanoparticle biostimulants (SA-CS NP) possesses a positive influence on enzymatic and antioxidative function in plants. The activity of the antioxidative enzymes enhanced at a significant rate. In maize, after two days of biostimulant application, SOD, POX and CAT activities has also been increased by two levels comparable with SA treated plants after different intervals of exposure [157].

Table 4. Effects of different types of biostimulants on plant cellular and physiological mechanisms and their benefits for agriculture and environment [17].

Biostimulants Source	Cellular Mechanism	Physiological Mechanism	Agricultural Benefits	Environmental Benefits	References
Humic acids	Induce proton pumping, ATPases activity promote cell elongation and cell wall loosening	Enhanced accumulation of root biomass	Enhanced nutrient efficiency and root foraging ability	Improved yield and reduced utilization of fertilizers	[159]
Seaweed extracts	Upregulation of micronutrient transports encoding gens by application of <i>A. nodosum</i> (<i>Brassica napus</i>)	Increased root mass and mineral uptake	Enhanced accumulation of minerals in plant tissue	Biofortification of micronutrients (Mg, Fe, Cu, Zn)	[160]
Protein hydrolysate	Stimulation of biosynthesis of phenylalanine ammonia-lyase (PAL) via. enzymatic hydrolysis of alfalfa (<i>Medicago sativa</i>) and accumulation of flavonoids under salt stress	Protection against oxidative damage and UV rays	Improved crop resistance to abiotic stress (salt stress)	Improved crop yield under stress conditions (high salinity)	[153,161]
Glycine betaine	Protection against salt induced photodamage in quinoa	Maintenance of photosynthetic activity under salinity	Improved crop resistance to abiotic stress (salt stress)	Improved crop yield under stress conditions (high salinity)	[154,162]
Plant growth promoting Rhizobacteria	Induced auxin signaling pathways in roots via. application of <i>Azospirillum brasilense</i> wheat (<i>Triticum aestivum</i>)	Improved root mass and intensity	Enhanced nutrient efficiency and root foraging ability	Improved yield and reduced utilization of fertilizers	[152]

The application of *Moringa oleifera* based extract contributed to a decline in the antioxidant enzyme function in plants (CAT, POX, and SOD). Simultaneously, total phenolic and ascorbic acid content were significantly higher at increasing concentration of biostimulants [156]. Aqueous extract of garlic enhanced tomato oxidation properties. The activity of the SOD significantly increased in comparison to concentration of garlic extract. With foliar spray of biostimulants at a concentration of 200 $\mu\text{g L}^{-1}$, the maximum enzyme activity was detected; the POX activity was also maximum after the biostimulant application. A lower concentration of aqueous garlic extract (50 kg L^{-1}) did not alter the enzymes activities [163]. Sunflower (*Helianthus annuus* L.) soaked seed in 3% maize seed based extract and application at 1 mM magnesium dose, the antioxidant potential of sunflower was triggered. POX, CAT, and SOD enzyme activities increased by 65.3, 76.9, and 83.2%, respectively, in comparison to the control. The excess concentration of antioxidative enzymes was connected with the foliar spray of the magnesium ions under which the intensity of photosynthetic process was enhanced [164]. Biostimulants also improved the activity of enzyme phenylalanine ammonia lyase (PAL). Although PAL activity (7.9 to 0.22 $\text{U ml}^{-1} \text{min}^{-1}$) in the control was 0.4%, it increased up to 10 times in treated plants (9.0 to 0.01, 9.7 $\text{U ml}^{-1} \text{min}^{-1}$). PAL, an enzyme of secondary metabolism catalyzes the *trans*-elimination of ammonia for phenyl compounds synthesis. A higher concentration of phenolic compounds was also reported under stress condition. It was concluded that biostimulants stimulate the plants to enhance the levels of secondary metabolites as defensive compounds [165]. Furthermore, organic wastes including plant residues, wood, and other waste residues can be used to produce biostimulants. The compost proved by the EU as reliable sustainable material must be therefore, primarily composed of natural products based raw materials, and distinguished via minimal concentration of heavy metals and toxic compounds. The processed compost products must be free from infectious agents (*E. coli* and *Salmonella* spp.) [166]. In the production of red lettuce, agro-industrial compost seemed to be an alternative way to peat. The compost enhanced the antioxidant activity in leaves of lettuce. During the early autumn, compost-grown lettuce leaves produced 1.5-fold more antioxidants than grown in the hot summer and also higher than autumn grown peat crop [167].

6. Role of Biostimulants on Nutrient Use Efficiency of Plants

A valuable tool in improving the availability of soil nutrients, incorporation, and assimilation can easily be achieved by the application of bioactive natural products and microbial inoculants [12]. Increased productivity of resource usage is very important for environmental and economic concerns [1]. The use of legume-based PH, particularly as substrate drench, strengthened the leaf number, SPAD index (Soil Plant Analyzing Development), and foliage production in greenhouse tomatoes under optimal and suboptimal N regions (110, and 6 mg/L , respectively) [168]. Improve agronomic responses of the tomato treated with PH was coupled with the root stimulation that facilitated N absorption and translocation. In addition, the PH application was improved under suboptimal N concentrations by up-regulation of expression of genes which are considered to be active in N assimilation for amino acids transporters and ferredoxin-glutamate (NADH/NADPH dependent) and glutamine synthesis in the roots. In contrast, Fiorentino et al. [167] investigated the biostimulant behavior of the two *Trichoderma* strains (*T. virens* GV41 or *T. harzianum* T22) under ideal growth conditions of N availability in two leafy vegetables, rocket, and lettuce. They observed that *T. virens* GV41 improved NUE in lettuce and promoted the native N uptake both in vegetables and soil. The effect was more pronounced in lettuce in a species-dependent manner. The results also revealed that inoculation from *Trichoderma* significantly modulated the structure of rhizosphere eukaryotic populations by providing considerable impacts with suboptimal N in contrast with N fertilized treatments. Furthermore, bacterial inoculants promotes the nutrients availability and their mobilization by the plant. Koskey et al. [168] identified 42 rhizobial isolates from the soil for characterization of biochemical, morpho-cultural, and genetic traits in the modified root nodules of climbing bean cultivars. They identified the bacterial strains with apparent

biofertilizer properties capable of performing under stress conditions. The application of complex microbial inoculants comprising of genera, viz., *Azospirillum*, *Streptomyces*, *Bacillus*, *Trichoderma*, *Pseudomonas*, and *R. irregularis* were considered effective in wheat production in comparison to chemical fertilizers and the commercial minerals at the recommendation dose for field application distinguished by marginal P and N deficient soils [35]. The solubilization of zinc (Zn) by PGPR is practically a recent method to validate this method, a researcher team expertise not only screened but also analyzed the Zn-solubilizing rhizobacteria from sugarcane and wheat [169]. They found that microbial inoculants (*Enterobacter cloacae*, *Pseudomonas fragi*, *Pantoea* sp.) has tremendous potential as a microbe-based biostimulants to minimize the Zn deficiency under mineral deficit soil conditions.

7. Quality Commodity Ramification of Biostimulants for Agri-Production

The microbial and non-microbial PBs can alter primary and secondary metabolism of plant which lead to the formation and aggregation of secondary metabolites, necessary in human's diet [70,79]. Singh et al. [48] investigated the application of *Trichoderma harzianum* biofortified with spent mushroom substrate (SMS) and earthworm grazed to evaluate its effect on quality of tomato. The results showed that tomato fruit quality in respect of antioxidative activity, carotenoids, total soluble sugars, and flavonoids and total polyphenolic content as well as minerals (Ca, Mg, K, P, Zn, and Mn) significantly increased by their application. In addition, Trejo-Téllez et al. [170] studied the effects of solar radiations, phosphates and phosphites at low or optimal level in the two mustard species: *Brassica juncea* and *Brassica campestris* to investigate the interactions on ratios of nitrate, flavonoids, and glucosinolates. The authors have noted that administration of phosphite in nutrient medium improves the scarcity of phosphate; thus, promotes the accumulation and synthesis of certain targeted glucosinolates and flavonoids in order to cope with nutritional stress.

Several researchers [171–174] studied the effect of exogenous PBs on functional and nutritional aspects of fruit trees and grapevines. Biostimulant-based products such as, PH, seaweed extract (*A. nodosum*), and vitamins (i.e., B-group) has insignificant effect on the quality of apples (size, strength of flesh, acidity, and total sugars), while red coloration was improved and increased in “Jonathan” apples harvested during the initial two year trials [173]. Likewise, selenium (Se) foliar spray on olives enhanced the functional and nutritional traits of extra virgin olive oil; besides, Se-biofortification, enhanced accumulation of antioxidant compounds was also recorded [174]. In their report, extra virgin olive oil itself have been benefited immensely from the up-regulated accumulation and synthesis and of potent antioxidative molecules, like phenols and carotenoids, which ultimately enhanced its oxidation capacity and subsequently its shelf life. The foliar application of three brassinosteroid analogs (Lactone, Triol, and 24-epibrassinolide) in a dose-dependent manner at the onset of véraison enhanced the color, anthocyanin pigment and total soluble solids with no impact on yield in “Redglobe” table grape [174]. In accordance with the previous research, the foliar spray of abscisic acid as biostimulant at a range of concentrations (200 and 500 mg/L) and the time period (7th and 21st days after ripening) promoted the formation of flavonoids and anthocyanin pigment in table grapes (*Vitis vinifera* × *V. aobrusca*) [173]. They also reported that ascorbate at 500 mg/L concentration leads to improvement in (i) anthocyanin accumulation of the individual and total, (ii) gene expression of *UFGT*, *CHI*, *F3H*, *DFR* biosynthetic genes and (iii) upregulation of transcription factors *VvMYBA1* and *VvMYBA2* [172].

8. Legal Framework and Limitation of using Biostimulants

Biostimulants are defined by what they do, not by what they are. The definition of PBs comes from the EBIC and is highly recommended because for the first time, there exists an official consensual definition as to what a biostimulant product is and how it can contribute to crop production. This is one of the novelties of the new regulation on

PBs use. Even though its effective entry in force is not scheduled till 2022, its importance justifies looking in detail at the changes that the new regulatory framework will bring to the agriculture sector. Biostimulants have been included in the new European Fertilizing Products Regulation. Now that the inclusion of biostimulants in the new law has been agreed, the key to understanding why matters have reached their present status lies in the need to harmonize what to date has been an excessively broad and diffuse regulatory framework.

The diversity of laws at national and international level existing till now has led to uncertainty among manufacturers when it comes to positioning biostimulants on markets in terms of their functions, as these were not clearly defined within any of today's legal frameworks. Such a lack of clarity has also affected farmers, who have lived with doubts and a lack of confidence in regard to which products are the most suitable for their needs. The new European Fertilizing Products Regulation sets out a new procedure for authorizing biostimulants in agriculture, which are now required to undergo a conformity assessment process by accredited bodies in each member state. This assessment of conformity will guarantee that biostimulants bearing the EU marking that come onto the market do so in full compliance of all legal requisites, thus affording farmers greater reassurance and better yield. The new regulation also includes stricter rules in respect of labelling of biostimulant products. Manufacturers can only declare those benefits derived from their products that have been scientifically proven; i.e., labels will only be allowed to mention benefits relating to improved efficiency of nutrient use, enhanced tolerance to abiotic stress, better crop quality traits, improved availability of confined nutrients in the soil, and rhizosphere or phyllosphere. In practice, these new requirements will provide greater transparency and confidence when defining the limits of the efficacy of biostimulants.

9. Future Insights and Challenges

Plant biostimulants represent a possible new class of agriculture income, complement agro-chemicals, such as pesticides and fertilizers, enhance abiotic stress resilience and increase productivity of agricultural commodities. The characterization of the PB bioactive components and the elucidation of molecular and physiological pathways of stimulation are of great importance for the science and commercial communities. The exploitation of small and efficient high-throughput phenotyping techniques is perhaps the most powerful strategy to innovate new PBs due to diverse matrices with various bioactive groupings and signaling molecules [28]. Ugena et al. [173] reported that high-throughput multi-trait screening is relevant in order to classify new possible biostimulants and characterize their mechanisms of action under both ideal and insufficient conditions (i.e., salinity). The findings showed that the mechanism behind the action in PBs are outlined in three classes utilizing this new technology: (i) the plant growth promoters, (ii) the stress relievers, and (iii) joint intervention. Likewise, Paul et al. [174] testified high-performance techniques like metabolomics, transcriptomics, and phenotyping which help to screen novel bioactive components and signaling molecules with biostimulatory characteristics and would provide agro-morphological and metabolomics features, underlying the protein hydrolysates effect on tomato. Further, Rouphael and Colla [97] proposed that to concentrate solely in the foreseeable future, the prominent stakeholders of biostimulants, viz., scientists and private industry must concentrate on creating a second version of PBs (biostimulant 2.0), with special biostimulatory synergistic steps to make agriculture more competitive by implementing microbial, non-microbial and natural PBs. Researchers have been functioning in the direction of finding novel applications of NUE and RUE to maximize their effects [2].

10. Conclusions

Biostimulants are the naturally occurring preparations that facilitate the seedling establishment and cultivation of fruit and vegetables. Although a positive impact of biostimulants has indeed been reported on extensively for the last several years, they are seldom included in standard technological innovations. This is related to farmers'

awareness of the functions and use of biostimulants which contributes to concerns of a rise in cultivation costs and a decline in plant quantity and quality ultimately affecting the crop profitability. The question is still the complexity of biopreparations and the need to prioritize suitable PBs or establishment of biostimulant seedlings to achieve the higher yields as well as quality for a particular plant variety. The market demands for the development of biopreparations with a multitude of services, which are convenient to use and coupled with other agents.

The industrialization of biostimulants based-seedlings has reduced the amount of chemical fertilizers in the ecosystem, and thus proved eco-friendly, reduce soil, air, and water pollution. In the case of global warming, this is particularly important. On average, agricultural production caters to 21% of the growing population. Global agriculture sector contributes an average of 22% with worldwide greenhouse effect, with the impact of chemical fertilizers at about 13%. The revolutionary new biostimulant based-seedling practices could make a considerable impact on environmental protection, but are mostly strongly aligned with sustainable agricultural and horticultural cultivation in order to produce inexpensive, easily accessible and nutritionally-aided food products. The impact of biostimulants is dependent on a variety of factors including the unprocessed products and the methods that led to crop species, processing conditions, and the climate. The essence of its beneficial effects, however, is not well known, so their modes of action in certain cases are still a challenge and must be recognized. Henceforth, biostimulants seedlings are still among the emerging trends in agriculture sector and require extensive study. Particular attention should be paid to the profound contribution of microorganism consortia and plant hydrolysates on crop growth and yields. The antioxidant capacity of plants treated with algae-containing biostimulants is also significant. Positive repercussions on plant quality and efficiency, no negative effects on human beings, animals or the ecosystem, increase biodiversity and enhancement of the soil characteristics are the major aspects of biostimulants seedlings applications.

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