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The role of biostimulants and bioeffectors as alleviators of abiotic stress in crop plants

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Abstract

The use of bioeffectors, formally known as plant biostimulants, has become common practice in agriculture and provides a number of benefits in stimulating growth and protecting against stress. A biostimulant is loosely defined as an organic material and/or microorganism that is applied to enhance nutrient uptake, stimulate growth, enhance stress tolerance or crop quality. This review is intended to provide a broad overview of known effects of biostimulants and their ability to improve tolerance to abiotic stresses. Inoculation or 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. Non-pathogenic bacteria capable of colonizing roots and the rhizosphere also have a number of positive effects. These effects include higher yield, enhanced nutrient uptake and utilization, increased photosynthetic activity, and resistance to both biotic and abiotic stresses. While most biostimulants have numerous and diverse effects on plant growth, this review focuses on the bioprotective effects against abiotic stress. Agricultural biostimulants may contribute to make agriculture more sustainable and resilient and offer an alternative to synthetic protectants which have increasingly falling out of favour with consumers. An extensive review of the literature shows a clear role for a diverse number of biostimulants that have protective effects against abiotic stress but also reveals the urgent need to address the underlying mechanisms responsible for these effects.

Keywords: Abiotic stress, Biostimulants, Bioeffectors, Microbial inoculants, Humic acid, Fulvic acid, Protein hydrolysates, Amino acids, Seaweed extracts, Bioprotection

Introduction

Plant biostimulants, sometimes referred to as agricultural biostimulants, are a diverse classification of substances that can be added to the environment around a plant and have positive effects on plant growth and nutrition, but also on abiotic and biotic 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 [1]. Biostimulants are not nutrients per se; instead they facilitate the uptake of nutrients or beneficially contribute to growth promotion or stress resistance [2]. A newly emerged paradigm emphasizes that plants are not standalone entities within their environments; instead they are host and partner to microorganisms of bacteria and fungi; plants are a host to numerous microbiota and those associations, both outside and within its tissues, allow them to respond and adapt to abiotic and biotic stress [3]. Reasonably, if we functionally optimize these associations, we may strengthen their role in plant stress protection.

The industry definition of biostimulants was originally proposed in 2012 and stated: "Plant biostimulants contain substance(s) and/or microorganisms whose function when applied to plants or the rhizosphere is to stimulate natural processes to enhance/benefit nutrient uptake, nutrient efficiency, tolerance to abiotic stress,

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and crop quality. Biostimulants have no direct action against pests, and therefore do not fall within the regulatory framework of pesticides". Biostimulants were loosely defined for a long time and often regarded dubiously because of their aggregate nature and the inherent difficulty to determine which specific components were making beneficial contributions. The definition proposed by du Jardin [1] "A plant biostimulant is any substance or microorganism applied to plants with the aim to enhance nutrition efficiency, abiotic stress tolerance and/or crop quality traits, regardless of its nutrients content" represents the clearest and most concise way to define biostimulants.

Our understanding of biostimulants and their potential effects has been expanding at a considerable rate [4]. The role of biostimulants, specifically in regard to growth promotion and nutrient availability, has been reviewed (du Jardin [1, 4–6]). In addition to numerous general reviews, many categories of specific biostimulants have been extensively reviewed such as protein hydrolysates [7], seaweed extracts [8], silicon [9], chitosan [10], humic and fulvic acids [11], the role of phosphite [12], arbuscular mycorrhizal fungi [13], *trichoderma* [14], plant growth-promoting rhizobacteria [15]. These reviews have focused on plant growth promotion and biotic stress but our intent with this review is to comprehensively address what is known about biostimulants ameliorating the effects of abiotic stress (Table 1). The majority of these studies were conducted as greenhouse or field experiments. The literature has mainly focused on crop species with a large representation of cereal crops such as wheat, barley, and corn. Finally, we attempted to map different categories vs. their physiological function in plants.

Algal extracts

Seaweed extracts (SWE) as biostimulants are emerging as commercial formulations for use as plant growth-promoting factors and a method to improve tolerance to salinity, heat, and drought. Algal extracts target a number of pathways to increase tolerance under stress (Fig. 1). Seaweeds are red, green, and brown macroalgae that represent 10% of marine productivity [8]. Macroalgae have been used as organic fertilizers for thousands of years and are still in use [64]. Currently, there are over 47 companies producing and marketing various algal extracts for agricultural use; the majority of the formulations are from the brown algae, *Ascophyllum nodosum* [65].
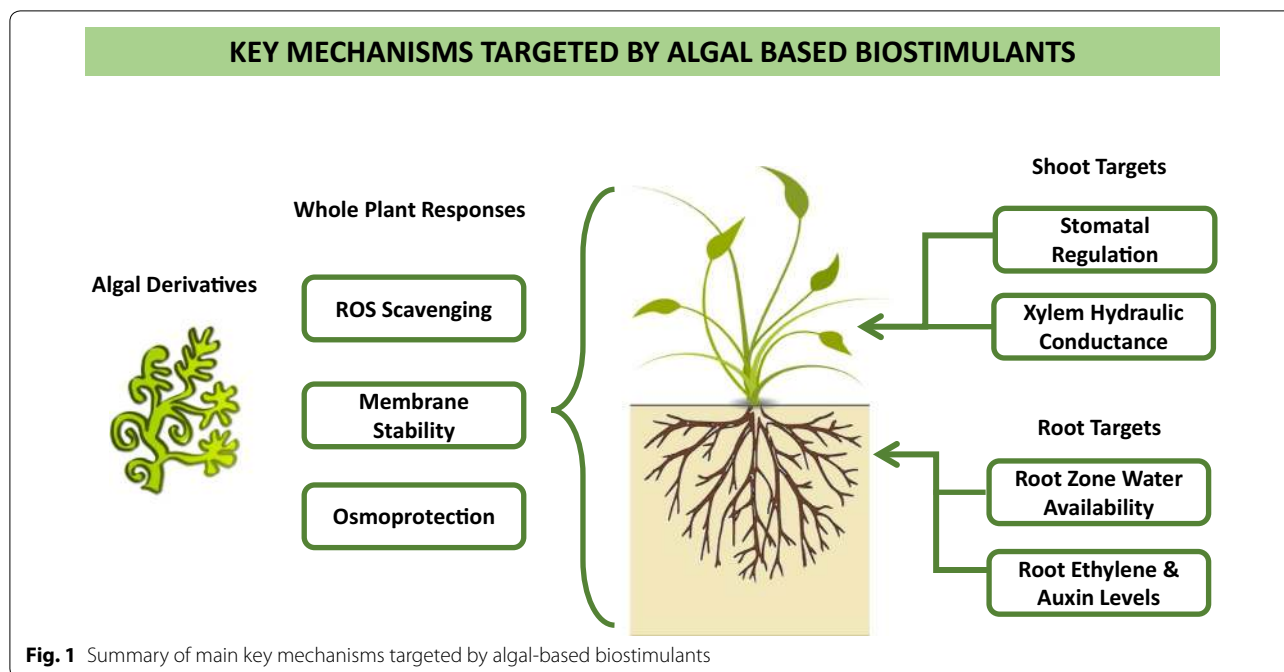
While the growth-promoting effects of seaweed extracts have been documented in many species [8, 66], very little is actually known about the mechanisms behind these effects. The variable and complex nature of these substances makes it difficult to determine exactly which components are playing a key role. Commercial formulations of SWEs are often proprietary, and the composition is largely dependent on the method of extraction. Indeed, characterization of the actual composition of most common algal-based commercial products would be useful first step to better hypothesize and/or depict a cause–effect relationship of their mechanism of action. Mechanical disruption, pulverization, acid or alkali extractions are some of the more common methods employed [8]. Most commercial products are derived from red (ex *Lithothamnium calcareum*) and brown (ex *Ascophyllum nodosum*, *Durvillaea potatorum*) macroalgae [67]. The role of SWEs and cold tolerance is now emerging. Very recent work has focused on SWEs and their ability to enhance tolerance to chilling stress. When multiple extracts were tested for their ability to enhance cold tolerance in maize only extracts rich in Zn and Mn were able to enhance tolerance through enhanced ROS responses. In this case, the protective effects likely stem from supplying plants with micronutrients that play a role as co-factors in anti-oxidative enzymes [63]. These results indicate that nutrient deficiency stress induced by cold can be overcome by supplying SWEs rich in micronutrients to improve oxidative stress tolerance. Previous studies with corn seedlings under root chilling stress supplemented with micronutrients demonstrated the utility of nutrient seed priming [68].

Some work has been done in model systems with the goal of determining the physiological and molecular responses induced by SWEs. In order to better understand the active components of *A. nodosum*, Rayirath et al. [55] separated the organic-sub-fractions of extracts and tested them with *Arabidopsis thaliana* and freezing experiments. Plants grown in vitro with sub-fractions added to the substrate or in "Peat pellet freezing assays" irrigated with sub-fractions were tested for freezing tolerance. The authors found that the ethyl acetate extracted fraction, rich in fatty acids and sterols enhanced freezing tolerance over water treated (controls) at temperatures from −2.5 to −5.5 °C. Treated plants maintained faster rates of recovery, greater membrane integrity, and had 70% less chlorophyll damage upon freezing recovery as well as increased expression of key freezing tolerance genes such as RD29A, COR15A, and CBF3 [55]. Priming of key tolerance genes prior to exposure to stress greatly increases tolerance in many cases. The lipophilic components were found to be rich in fatty acids such as butyric acid, palmitic acid, oleic acid, linoleic acid the sterol fucosterol. These extracts increased proline content and total soluble sugars, contributing to freezing tolerance [56]. *A. nodosum* extracts have even been used to reduce cold stress sensitivity in *Kappaphycus alvarezii*. *Kappaphycus alvarezii* is a red algae and the most important source of carrageenans; which are hydrophilic colloids

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Table 1 Summary of species, biostimulant, and stress effect

Type of BE	Crop	Stress and effect	Reference
A. brasilense	*T. aestivum*	Drought tolerance	[16, 17]
A. brasilense	*C. arietinum*	Salt tolerance	[18]
A. brasilense	*V. faba*	Salt tolerance	[18]
A. brasilense	*L. sativa*	Salt tolerance	[19, 20]
A. brasilense	*T. aestivum*	Salt and osmotic stress	[21]
A. brasilense	*L. lycopersicum*	Drought tolerance	[22]
A. brasilense/P. dispersa	*C. annuum*	Salt tolerance	[23]
A. chrococcum	*Z. mays*	Salt tolerance	[24]
A. chrococcum	*T. aestivum*	Salt tolerance	[25]
A. chrococcum	*T. aestivum*	Temperature tolerance	[26, 27]
A. lipoferum	*T. aestivum*	Salt tolerance	[28]
A. nodosum	*Kappaphycus alvarezii*	Cold tolerance	[29]
A. nodosum	*P. dulcis*	Ion homeostasis	[30]
A. nodosum	*C. sinensis*	Drought tolerance	[31]
B. phytofirman,	*Vitis vinifera*	Cold tolerance	[32, 33]
F. glaciei	*Solanum lycopersicum*	Cold tolerance	[34]
Fulvic and humic acids	*F. arundinacea*	Drought tolerance	[35, 36]
Fulvic and humic acids	*A. palustris*	Drought tolerance	[37]
Glycinebetaine	*L. lycopersicum*	Chilling stress	[38]
H. diazotrophicus	*H. vulgare*	Salt tolerance	[39]
Humic acid and phosporous	*C. annuum*	Salt tolerance and ion homeostasis	[40]
Humic acids	*O. sativa*	Oxidative and drought stress	[41]
Humic acids	*P. vulgaris*	Salt tolerance	[42]
Megafol	*L. lycopersicum*	Drought tolerance	[43]
Melatonin	*Z. mays*	Chilling tolerance	[44]
P. frederiksbergensis	*Solanum lycopersicum*	Cold tolerance	[34]
P. putida	*T. aestivum*	Heat tolerance	[45]
P. putida	*S. bicolor*	Heat tolerance	[46]
P. vancouverensis	*Solanum lycopersicum*	Cold tolerance	[34]
P.dispersa	*T. aestivum*	Cold tolerance	[47]
Protein hydrolysates	*H. vulgare*	Ion homeostasis	[48]
Protein hydrolysates	*Z. mays*	Salt tolerance	[49]
Protein hydrolysates	*T. aestivum*	Heavy metal tolerance	[50]
Protein hydrolysates	*L. sativa*	Salt tolerance, cold tolerance	[51, 52]
Protein hydrolysates	*D. kaki/D. lotus*	Salt tolerance	[53]
Protein hydrolysates	*Lolium perenne*	Heat tolerance	[51]
R. leguminosarum	*V. faba*	Salt tolerance	[54]
R. leguminosarum	*P. sativum*	Salt tolerance	[54]
SWE	*A. thaliana*	Cold tolerance	[55, 56]
SWE	*P. pratensis*	Salt tolerance	[57]
SWE	*A. stolonifera*	Heat tolerance	[58]
SWE	*S. oleracea*	Drought tolerance	[59]
SWE	*L. sativa*	Ion homeostasis	[60]
SWE	*V. vinifera*	Drought tolerance and ion homeostasis	[61]
SWE	*S. nipponica*	Drought tolerance	[62]
SWE	*P. eugenioides*	Drought tolerance	[62]
SWE	*Z. mays*	Cold tolerance	[63]

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KEY MECHANISMS TARGETED BY ALGAL BASED BIOSTIMULANTS

Whole Plant Responses

Algal Derivatives

ROS Scavenging

Membrane Stability

Osmoprotection

Shoot Targets

Stomatal Regulation

Xylem Hydraulic Conductance

Root Targets

Root Zone Water Availability

Root Ethylene & Auxin Levels

Fig. 1 Summary of main key mechanisms targeted by algal-based biostimulants

largely used in foods and dairy products [29, 69]. Algal extracts have also been used on Kentucky bluegrass (*Poa pratensis* L. cv. Plush) to alleviate salinity stress from saline watering in turfgrass experiments [57]. Similarly SWE-based cytokinins have been used on creeping bentgrass (*Agrostis stolonifera* L.) to improve tolerance to heat stress [58]. SWEs from *A. nodosum* have also been used for ornamental plants, such as *Spiraea nipponica* "Snowmound" and *Pittosporum eugenioides* "Variegatum", to enhance drought tolerance. Treated plants showed higher phenolic, proline, and flavonoid content while demonstrating improved physiology under mild drought stress conditions [62].

In horticultural crops and trees, SWE have been largely used for similar purposes. *A. nodosum* SWE increased RWC, Fresh Weight, and Dry Weight in spinach (*Spinacia oleracea* L.) plants under drought stress with some adverse effects on the nutritional value through reduced ferrous ion chelating ability [59]. SWE applied to seedlings of lettuce (*Lactuca sativa* L.) enhanced cotyledon growth similar to fertilization with potassium [60].

Foliar application of marine bioactive substances (isopropanol extracts from microalgae) to grape plants (*Vitis vinifera* L) increased leaf water potential and stomatal conductance under drought stress [61]. Consistent with an improved stomatal response, it was also observed that K^+ and Ca^{2+} fluxes at the stomatal level were higher in treated plants. Commercial formulations of *A. nodosum* have been tested on almond plants (*Prunus dulcis* [Mill.] D. A. Webb), which demonstrated increased growth and

accumulation of K^+. In conditions with ample K^+ both MegaFol and GroZyme (Valagro, Atessa, Chieti, ITALY) increased leaf area and number of leaves greater than controls treated with water or K^+. In K^+-deficiency conditions only MegaFol and a foliar application of K^+ was able to stimulate growth, although at lower levels than observed with adequate K^+ nutrition [30]. Accumulation of K^+ is an essential step in protecting against both ionic and osmotic stress and may contribute to tolerance. Orange trees, *Citrus sinensis* L., subjected to drought stress and treated with commercial extracts of *A. nodosum* had better water relations and increased water use efficiency (WUE) under irrigation at 50% restitution of evapotranspired water [31]. The promise of biostimulants to increase drought tolerance and WUE holds great potential for drought prone regions where horticultural crops and fruit trees are agronomically important but water availability is becoming less reliable due to urbanization and climate change.

As earlier noted, almost all of the above-mentioned experiments with SWE use commercial formulations. This may be of some concern, due to the variable nature of these products and formulation methods. A recent transcriptomic study using *A. thaliana* plants treated with two different commercial *A. nodosum* extracts showed that not all extracts are alike. One commercial product resulted in dysregulation of 4.47% of the transcriptome while the other extract only affected 0.87% [70]. Since transcriptional priming is likely a key component in enhancing abiotic stress tolerance using SWEs,

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these differences imply significant variability in responses elicited. Compositions of the extracts differed greatly, indicating that choice of commercial product may have a significant effect on plant responses. Commercial formulations are often proprietary and the exact composition and extraction methods, shifting the burden to the research community to analyse and isolate the active components in these products. In order to identify and characterize how these SWEs affect plants, some form of standardization is necessary.
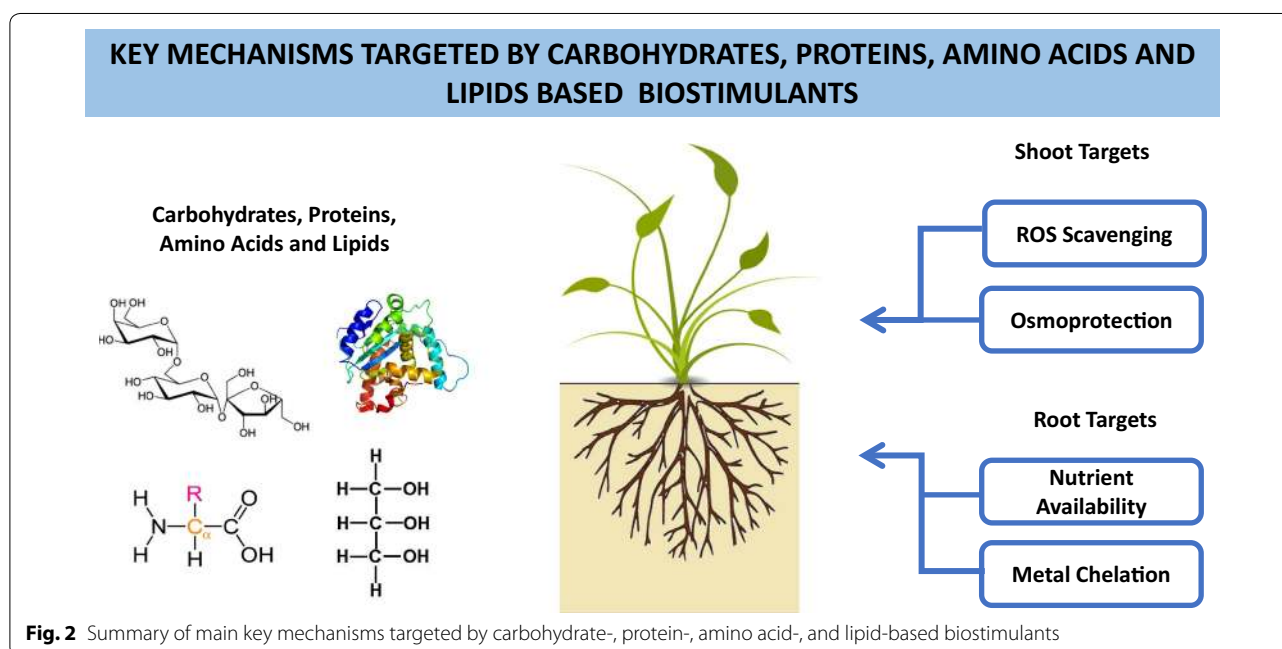
Carbohydrates, proteins, amino acids, and lipids

Protein hydrolysates are mixtures of polypeptides, oligopeptides, and free amino acids derived from partial hydrolysis of agricultural by-products from animals and plants [7]. Carbohydrates, proteins, amino acids, and lipids may increase stress tolerance through different (Fig. 2). The effects of amino acids on ion fluxes across membranes have been clearly established, with most having a positive effect on reducing NaCl-induced potassium efflux [48]. Protein hydrolysates (PH) are often sold as formulations that include plant growth regulators. The bulk of PH products, over 90%, are produced from chemical hydrolysis of animal by-products while enzymatically processed plant-based products are a recent development [7].

Megafol (Valagro, Atessa, Chieti, ITALY) is a commercial biostimulant comprising vitamins, amino acids, proteins, and betaines from plant and algal extracts. Application of Megafol to tomato plants under drought stress enhanced induction of a number of drought responsive genes such as tomato orthologs of *RAB18* and *RD29B*. Treated plants also had higher fresh weight and relative water content under drought stress, indicating a protective effect on water status and stress responsive genes [43, 71]. When hydrolysate-based biostimulants from alfalfa (*Medicago sativa* L.), containing triacontanol (TRIA) and indole-3-acetic acid (IAA), were applied to maize plants under salt stress, the protective effects were amplified. Treated plants had higher flavonoid, proline, and potassium content in salt stress conditions over untreated controls [49]. Extracts that are rich in amino acids may play a role in increasing cold tolerance. When lettuce plants (*Lactuca sativa*) were treated with an amino acid mixture, derived from enzymatic hydrolysis of proteins, (Terra-Sorb) and subjected to cold, treated plants had higher fresh weights and improved stomatal conductance [51]. Use of animal derived amino acid hydrolysates on strawberry plants after transplantation and cold stress did not improve survival though some growth promotions were observed in the absence of stress [72]. Perennial Ryegrass (*Lolium perenne* L.) treated with hydrolyzed amino acids and high temperatures (36 °C) had improved photosynthetic efficiency over control plants [51].

Mutants of *A. thaliana* deficient in production of proline have stress sensitive phenotypes [73]. These plants can have their phenotype rescued with exogenous application of L-proline, a common amino acid available in biostimulant formulations of various amino acids and hydrolysate mixtures [74]. Hydrolysates from wheat germs show strong anti-oxidant and free radical scavenging properties as well as the ability to chelate some metals [50].

Fig. 2 Summary of main key mechanisms targeted by carbohydrate-, protein-, amino acid-, and lipid-based biostimulants

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Lettuce (*Lactuca sativa* L.) is particularly salt sensitive and the addition of plant-derived protein hydrolysates improved fresh yield, dry biomass, and root dry weight as well as increased concentrations of osmoyltes, glucosinolates and the composition of sterols and terpenes [52]. Hydrolysates have applications for trees, which require considerable investment costs and can be vulnerable to drought. Japanese persimmon trees, *Diospyros kaki* L. cv. "Rojo Brillante" grafted on *Diospyros lotus* L., are particularly sensitive to drought stress [53]. Treatment of these trees with calcium protein hydrolysates decreased chloride uptake under saline irrigation, lowered water potentials as well as increased the concentration of compatible solutes [53], all of which would enhance plant growth under saline stress.

Recent reports indicate that melatonin, derived from L-tryptophan via the shikimate pathway, can prime seeds to tolerate adverse environmental conditions at imbibition and germination stages [75]. Corn seeds pre-treated with melatonin show increased tolerance to chilling stress upon germination, indicating a priming effect by melatonin [44]. Melatonin may prove to be an effective biostimulant for improving stress tolerance of seedlings.

Glycinebetaine is a compatible solute accumulated in many plants in response to salt stress [76]. Exogenous application of glycinebetaine has increased tolerance for environmental stresses such as drought, chilling, freezing, salinity, and oxidative stress. Foliar application of glycinebetaine results in rapid uptake by leaves and concentration in meristematic tissues. Rapid uptake and localization of glycinebetaine in these most vulnerable tissues are particularly beneficial in chilling and freezing stress where glycinebetaine can exert a protective effect [77]. Transgenic plants of various species expressing two biosynthetic genes, *codA* and *betA,* produce more glycinebetaine and had an increased tolerance to abiotic stress [38, 78]. Exogenous application of small amounts of compatible solutes such as proline and betaine to barley roots resulted in an immediate reduction of NaCl-induced efflux of K^+, indicating that ion fluxes across the membrane can be affected by relatively low concentrations of compatible solutes [79]. The cause–effect relationship between accumulation of compatible solutes and stress protection still remains to be fully understood [80]. However, a better understanding of the specific mechanisms of action of these molecules is becoming increasingly important if we want to make predictions on which combination of biostimulants can be more effective.

Humic and fulvic acids

Humic and fulvic substances are the major organic components of lignites, soil, and peat. Humic and fulvic acids are produced by the biodegradation of organic matter r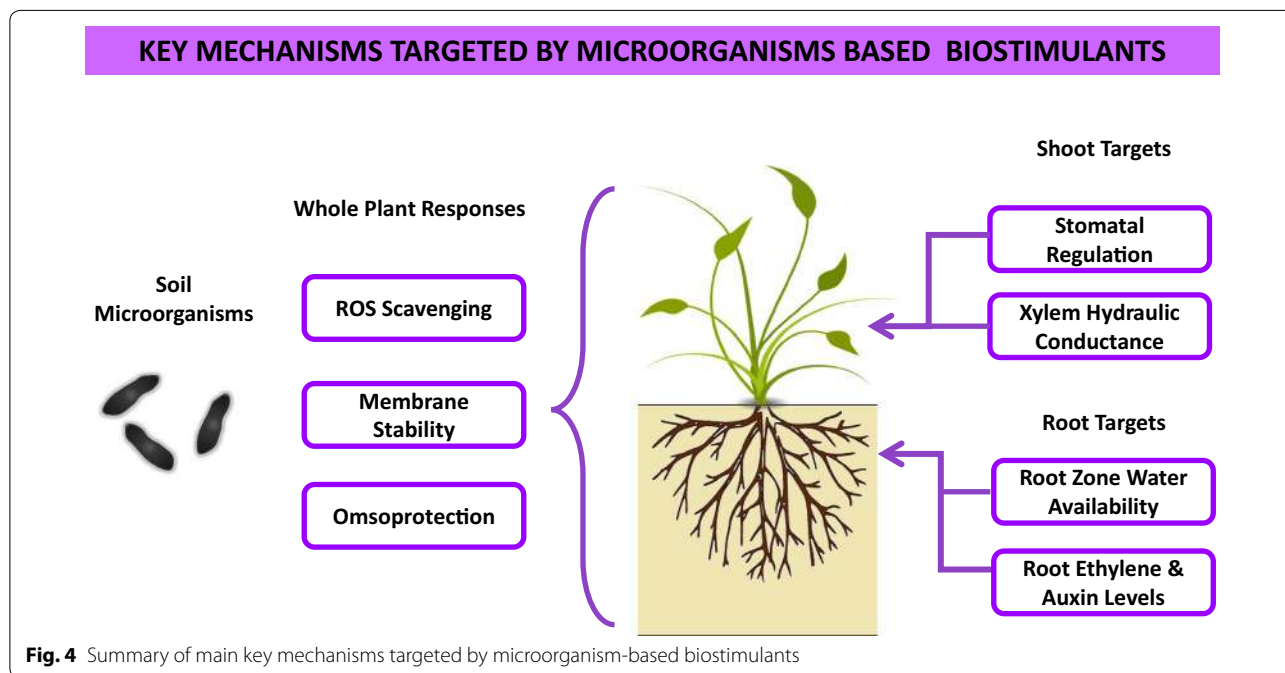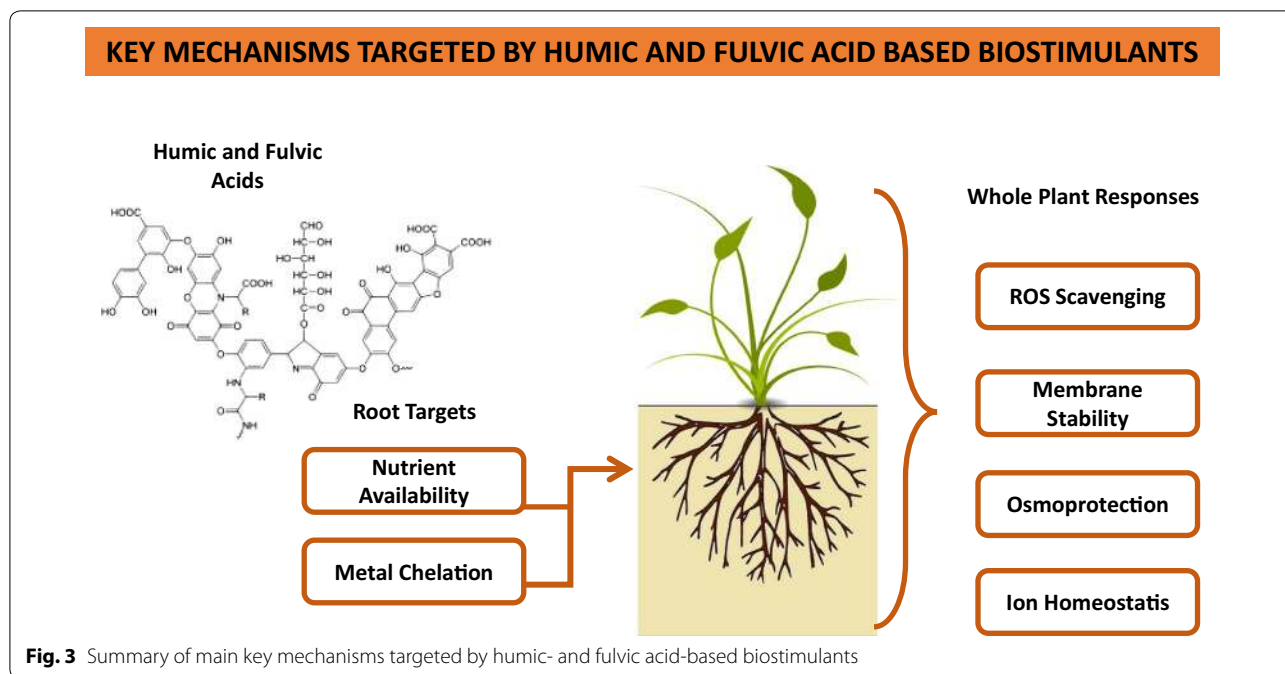esulting in a mixture of acids containing phenolate and carboxyl groups. Fulvic acids are humic acids with a higher oxygen content and lower molecular weight [81]. A number of examples exist indicating the potential for these substances to improve abiotic stress tolerance in plants (Fig. 3). Pre-treatment of tall fescue (*Festuca arundinacea* Schreb.) and creeping bentgrass (*Agrostis palustris* Huds. A.) with seaweed extract and humic acid increased leaf hydration under dry soil conditions as well as root growth, shoot growth, and antioxidant capacity [35, 58]. Further studies with bentgrass showed these extracts, high in cytokinins, combined with humic acid increased drought tolerance as well as endogenous cytokinin content [37].

Treatment of bell pepper (*Capsicum annuum* L. cv. Demre) with humic acid and phosphorous resulted in plants with reduced Na content and elevated N, P, K, Ca, Fe, Mg, S, Mn, and Cu ion contents in roots and shoots, which were associated with a general protective effect under mild salinity stress [40]. Application of humic acids to common bean (*Phaseolus vulgaris* L.) under high salinity (120 mM NaCl) increased endogenous proline levels and reduced membrane leakage [42], which are both indicators of better adaptation to saline envirnoments.

Humic acid extracts seem to be beneficial also for field crop monocots. Extracts from vermicompost applied to rice (*Oryza sativa* L.) played a role in activating anti-oxidative enzymatic function and increased ROS scavenging enzymes. These enzymes are required to inactivate toxic-free oxygen radicals produced in plants under drought and saline stress [41]. One possible mode of action for vermicompost may be the differential regulation of proton ATPases located in the vacuolar and plasma membranes. When Micro-Tom tomato plants were treated with vermicompost, plasma membrane proton extrusion was increased by over 40% which facilitated acid growth and nutrient uptake potential. Interestingly, the auxin insensitive mutant *diageotropica* (dgt) showed no increase in proton extrusion, indicating that humic substance may increase root growth through mediating auxin signalling [82].

Microorganisms affecting stress tolerance

While plants are known to establish symbiotic relationships with bacteria, our understanding of those relationships under abiotic stress is rudimentary. However, some of the targets of microorganisms that increase abiotic stress tolerance have been identified (Fig. 4). Bacteria with the potential to act as biostimulants have been isolated from a number of ecosystems with saline, alkaline, acidic, and arid soils. These bacteria belong to several genera such as *Rhizobium*, *Bradyrhizobium*, *Azotobacter*, *Azospirillum*, *Pseudomonas*, and *Bacillus*. Members of these genera have developed strategies to adapt and

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KEY MECHANISMS TARGETED BY HUMIC AND FULVIC ACID BASED BIOSTIMULANTS

Humic and Fulvic Acids

Whole Plant Responses

ROS Scavenging

Membrane Stability

Osmoprotection

Ion Homeostatis

Root Targets

Nutrient Availability

Metal Chelation

Fig. 3 Summary of main key mechanisms targeted by humic- and fulvic acid-based biostimulants

KEY MECHANISMS TARGETED BY MICROORGANISMS BASED BIOSTIMULANTS

Shoot Targets

Stomatal Regulation

Xylem Hydraulic Conductance

Whole Plant Responses

Soil Microorganisms

ROS Scavenging

Membrane Stability

Omsoprotection

Root Targets

Root Zone Water Availability

Root Ethylene & Auxin Levels

Fig. 4 Summary of main key mechanisms targeted by microorganism-based biostimulants

thrive under adverse conditions [83, 84]. Amongst these adaptations, alterations to the composition of the cell wall and the ability to accumulate high concentrations of soluble solutes are common. These allow for enhanced water retention and increased tolerance to osmotic and ionic stress. Cell wall composition is altered through enrichment for exopolysaccharides (EPS) and lipopolysaccharide–proteins and polysaccharide–lipids which my form

a protective biofilm on the root surface [85, 86]. Plant growth-promoting rhizobacteria (PGPR) inoculated soils can ameliorate plant abiotic stress responses. A number of recent reviews have extensively covered the protective effects of *Rhizobium* against abiotic stress in plants [87]. Most documented growth enhancement determined by these bacteria is associated with high level of IAA, which has been proven to alleviate salt stress [88]

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and EPS production that may help in maintaining a film of hydration around the roots and/or help re-establishing favourable water potential gradients under water limitations. These functions have been proven useful under saline stress [89], extremes of temperature, pH, salinity, and drought [87, 90]. Inoculation of maize with *Azotobacter* strains has been shown to have general positive effects under saline stress by facilitating uptake of K^+ and exclusion of Na^+ as well as increasing phosphorous and nitrogen availability [24]. In wheat, inoculation of salt tolerance *Azobacter* strains increased biomass, nitrogen content, and grain yield under salt stress [25].

Tolerance to salt stress varies within these microorganisms and their tolerance can confer advantages to the host relationship under stress conditions. When two legumes, pea (*Pisum sativum*) and fava bean (*Vicia faba*), were inoculated with two different strains of *Rhizobium leguminosarum*, a salt-tolerant (GRA19) and salt-sensitive (GRL19) plants inoculated with the salt-tolerant strain performed better under moderate salt stress [54]. The authors further found that pea plants had larger nodules and high levels of nitrogen fixation under salt stress when inoculated with GRA19, the salt-tolerant strain of *R. leguminosarum*. Similar results have been observed for non-symbiotic free-living soil bacteria that are capable of fixing nitrogen. *Azospirillum brasilense* is closely associated with the plant rhizosphere and can colonize the surface of roots. When chickpea (*Cicer arietinum* L.) and faba bean were inoculated with *A. brasilense*, they experienced enhanced nodulation by native rhizobia and greater tolerance to salt stress [18]. Another free-living nitrogen-fixing species, *Azotobacter chrococcum* A2 demonstrated salt tolerance. Inoculation with *A. chrococcum* has been shown to increase yields of pea, potato, rice, wheat, and cotton in saline-arid soils. Increased root length and shoot growth was also observed with inoculation [26, 27] with significant positive yield effects for wheat (from 2.8 to 3.5 t ha^{-1} when grown in conjunction with *A. chrococcum*) [26, 27].

In barley, *Hartmannibacter diazotrophicus* E19 (T) is capable of colonizing roots in saline conditions. Inoculation of roots in saline soil increased root and shoot mass significantly, 308 and 189%, respectively. Inoculated roots also had increased relative water content over three and a half times that of control plants [39]. High concentrations of salt can also be inhibitory to rhizobial bacteria. While certain strains of *R. leguminosarum*, such as *viciae* SAAN1, are very salt tolerant and able to withstand up to 0.34 M NaCl, they often show lower rates of nodulation in saline soils. These strains are often less competitive with natural rhizobial populations, however.

The stress protection of bacterial biostimulants to rainfed field crops can be of particular relevance under increasing temperatures foreseen by most climate change prediction models. Wheat inoculated with the thermo tolerant *Pseudomonas putida* strain AKMP7 significantly increased heat tolerance. Inoculated plants had increased biomass, shoot and root length, and seed size. ROS generation under stress treatment was also lessened, with lower levels of expression observed for ROS response genes such as superoxide dismutase, ascorbate peroxidase, and catalase [45]. Similar results have been found with sorghum and other *Pseudomonas putida* AKMP strains [46]. Psychrophilic (cold-adapted) microorganisms are capable of surviving in extreme conditions and their interactions with plants provide potential mechanisms for improving tolerance [91]. While many strains of soil bacteria with growth-promoting properties have been isolated from low-temperature conditions, few have been tested in conjunction with plants subjected to cold stress.

Wheat inoculated with the cold-tolerant plant growth-promoting bacteria *Pantoea dispersa* showed improved growth and nutrient uptake, likely due to the solublization of phosphorous and production of IAA [47].

Inoculation of soil with psychrotolerant (cold tolerant) bacteria can play a role in chilling tolerance. The psychrotolerant soil bacterium, *Burkholderia phytofirman*, is a plant-growth-promoting rhizobacterium (PGPR) that is capable of colonizing multiple plant species. *B. phytofirman* was shown to play a role in enhancing chilling tolerance in *Vitis vinifera* L. by increasing ROS scavenging metabolites and stress-induced genes. Inoculated plants also recovered faster from chilling stress, returning to normal metabolic levels more quickly than controls [33]. *B. phytofirman* inoculation also alters carbohydrate metabolism and accumulation while having a protective effect on net photosynthesis during cold acclimation and stress [32, 92].

Tomato plants (*Solanum lycopersicum* cv Mill) were inoculated with cold-tolerant strains of *Pseudomonas vancouverensis*, and *frederiksbergensis* as well as *Flavobacterium glaciei* that were isolated from agricultural fields during winter. Treated tomato seedlings were subjected to a week of chilling stress at 15C and inoculation three of these strains showed significantly reduced electrolyte leakage and ROS activity [34]. Improved stress tolerance and growth-promoting effects of microorganism treatments have been seen in other species also. Inoculation of lettuce (*Lactuca sativa* L., cv Mantecosa) seeds with *A. brasilense* increased germination in the presence of salt and demonstrated tolerance through higher total fresh and dry weights of plants at harvest [19]. Additional experiments studying these effects have shown increased biomass, chlorophyll, ascorbic acid content, antioxidant content, and post-harvest shelf life after being subjected

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to salt stress [20]. Sweet pepper (*Capsicum annuum* L.) inoculated with *A. brasilense* and *Pantoea dispersa* was not affected by moderate levels of salinization, up to 80 mM NaCl, while uninoculated control plants demonstrated lower DW starting at 40 mM NaCl [23].

Triticum aestivum cv. Buck Ombú inoculated with *A. brasilense* sp. 245 and subjected to salt stress (320 mM NaCl) and osmotic stress (20 and 30% PEG 6000) had higher FW, DW, and RWC than non-inoculated controls [21]. Analysis of phospholipids and fatty acid composition in inoculated wheat indicated that the distribution profiles of major root phospholipids are altered in inoculated plants, possibly contributing to the increased tolerance [16]. Wheat inoculated with *Azospirillum lipoferum* and irrigated with 80 mM NaCl had significantly higher leaf and root dry weight than uninoculated controls [28].

While the mechanisms by which *A. brasilense* confer tolerance to osmotic stress are not clear, some evidence indicates that inoculation induces wider xylem vessels and greater hydraulic conductance [17]. In inoculated tomato plants subjected to water stress similar changes have been observed, such as larger xylem vessel area, higher stem-specific hydraulic conductivity, thicker stems [22]. Pepper plants co-inoculated with *A. brasilense* and *Pantoea dispersa* accumulated more dry matter under salt stress. Inoculated plants showed higher stomatal conductance and rates of photosynthesis under salt stress. The chlorophyll concentration and efficiency of photosystem II were not affected in inoculated plants under stress conditions [23].

Inhibition of root growth under salt stress conditions is well documented. One of the primary causes of this inhibition is the production and perception of ethylene in the roots [93]. Plants and PGPR both have ACC-deaminases, which possess the ability to lower the concentration of ethylene in the roots and root zone. PGPR-derived ACC-deaminases can reduce ethylene induced inhibition by reducing root zone ethylene [94] and contribute to maintain relatively higher root-to-shoot ration, a trait that would result beneficial under water shortage.

Conclusions

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 is identified that may yield highest impact on stress protection; high priority should be given to better understanding of the causal/functional mechanism of biostimulants. Only once a good understanding of these mechanisms has been reached; we will be able to move to the next generation of biostimulants where synergies and complementary mechanisms can be functionally designed.

Comprehension of the specific mechanisms that should be potentiated to overcame a specific stress can be based today on sound/reasonable hypotheses and be more fruitful than the try-and-see approach. A comprehensive and systematic approach has been proposed to discover and characterize novel biostimulants and understand the mode of action for those both known and new using a combined approach utilizing biology, chemistry, and 'omics [95]. Meta-analysis of the effects of biostimulants has been proposed and an extensive meta-level examination of humic substances on plant growth has been conducted. The analysis found that humic substances increased the dry weight of shoots and roots by at least 20% [96]. However, it should be noted that the diverse conditions, compositions, and species tested do not lend to robust meta-level analysis when an excessive number of variables is present. Identification of synergistic/complementary properties of biostimulants can be pivotal to develop specific formulations targeted to enhance plant response to abiotic stress. For example, biostimulants for improving plant resilience in water limiting environments should stimulate root vs. 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 favourable 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 favour of the plants.

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