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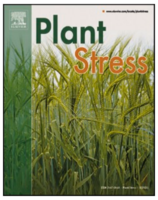

Review

Unravelling the nexus of plant response to non-microbial biostimulants under stress conditions

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ABSTRACT

Contemporary challenges facing the agricultural sector have garnered the interest of all stakeholders on the novel toolset of biostimulants. These products could serve as pivotal actors in the forthcoming transition toward ever more essential sustainable production practices. Regardless of their type, biostimulants have the potential to enhance resource efficiency while concurrently fortifying plant resilience to adverse abiotic stress factors. Recent research advances have fundamentally focused on assessing quantifiable parameters, largely overlooking the numerous and intricate biochemical, cellular, and metabolic interactions between plants and biostimulants. It is consequently not surprising that, to date, the mechanisms of action and basic biochemical processes underlying biostimulants' effects on plants remain enigmatic. Concerning non-microbial biostimulants, which are the subject of in-depth exploration in this review, their inherently diverse nature, comprising formulations containing a plethora of distinct bioactive molecules, significantly complicates the investigation of mechanisms implicated in their mode of action. It is for this reason that we have rather elected to meticulously examine the effects, particularly in suboptimal environments, of a) protein hydrolysates; b) algal extracts; c) humic acids; and d) silicon. The objective of this analysis is to gain a comprehensive understanding of how these substances operate within plants by interpreting both their genetic and metabolic impacts. Comprehensive understanding of these effects could substantially underpin the reliability of these agents and usher to the identification of ever more effective formulations.

1. Introduction

The agricultural sector is increasingly moving toward sustainable practices on a global scale, aimed at preserving and restoring critical habitats, improving soil health, and water resources quality (MacLaren et al., 2022). This represents a genuine paradigm shift, encouraged by the inclusion of the green transition in government agendas and increasingly demanded by consumers. Since the industrial revolution, agriculture has increasingly relied on chemical inputs to improve production and defend crops, without considering the negative effects on the environment and health of future generations (Buffagni et al., 2021; Delitte et al., 2021). In this context, one of the main priorities of agriculture will be to adopt more environmentally friendly production methods aimed at long-term ecological sustainability and high-quality food production (Ceccarelli et al., 2021). It is precisely this progressive global trend that has favored the growth of the agriscience market, in which biostimulants represent the flagship product (Ebinezer et al., 2020).

Although the term 'biostimulant' was coined already in the first half of the 20th century, researchers have only begun to study its effects on agricultural production in depth (Nephali et al., 2020; Yakhin et al., 2017). A greater understanding of these ecological tools, coupled with the need for safer and more sustainable alternatives to synthetic products, justifies the continued growth of their market position, estimated to reach more than $5.5 billion by 2026 Ma et al. (2022). The definition of biostimulant has continuously evolved and been reformulated over the years following the evolution of the scientific theoretical bases. Currently, the most recent definition of biostimulant has been established by the European Regulation 2019/1009 (EC, 2019), which defines them as *'substances and /or microorganisms whose function, when applied to plants or the rhizosphere, is to stimulate natural processes to improve and benefit nutrient uptake, nutrient use efficiency, tolerance to*

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abiotic stress and crop quality.' As mentioned earlier, the classification criteria for biostimulant products have been reviewed several times. According to du Jardin (2015) and Van Oosten et al. (2017), as well as Colla and Rouphael (2015), plant biostimulants can be divided into microbial, such as fungi and rhizobacteria that promote plant growth, and non-microbial, such as seaweed extracts, protein hydrolysates, humic substances, silicon, and other biopolymers.

Regardless of the type, from an agronomic perspective, the most important effect of biostimulants is the increase in yield, often attributed to a greater assimilation of nitrogen, carbon and sulphur, better photosynthetic performance, and an increase in free carbohydrates, amino acids, proteins, phenolic compounds, pigments, and enzyme activities (Ertani et al., 2013b; Jannin et al., 2013, 2012). The ability of biostimulants to enhance the resources use efficiency (RUE) is a fundamental aspect that guarantees yields comparable to or even superior to those of plants treated with high doses of chemical fertilizers (Ma et al., 2022). Furthermore, biostimulants may boost the immune system of plants, providing greater protection against biotic and abiotic stresses such as heat, cold, frost, mechanical and chemical stress, salinity, drought, and oxidative stress (Park et al., 2017; Van Oosten et al., 2017). Mitigating abiotic stresses is certainly the most sought-after effect of biostimulants, as these stressors can cause a reduction in yield of up to 50 %, a risk that is projected to increase in the future due to rapid and uncontrolled climate changes (Yakhin et al., 2017).

Although the promising potential of biostimulants have been highlighted by numerous studies, most of the formulations currently on the market rely on empirical descriptions of their agronomic effects, such as stress mitigation and improved production and quality performance (Van Oosten et al., 2017; Zaid et al., 2020). Therefore, it is increasingly urgent to generate new scientific knowledge to create a theoretical framework allowing the creation of biostimulant formulations supported by more solid scientific evidence. To achieve this goal, scientific research must focus not only on evaluating agronomic parameters but also on understanding the biochemical characteristics of biostimulants and the heterogeneous metabolic, cellular, and chemical interactions between the biostimulant and the plant (Nephali et al., 2020). Additionally, to ensure the regulation and commercialization of products, a thorough knowledge and understanding of each specific product' primary mode of action is necessary. However, for many biostimulants, both microbial and non-microbial, a well-defined mode of action and a precise biochemical target site are still lacking (Yakhin et al., 2017). Most scientific research has focused on the generic effects of biostimulant application, such as stimulation of the photosynthetic process or modulation of specific plant stress signaling pathways, without delving into more general molecular and biochemical metabolic network modelling . This has led to a lack of clarity on the mechanism of action of most biostimulant products (Yakhin et al., 2017). It is essential for the development and implementation of a biostimulant industry based on solid scientific foundations to abandon empirical and non-repeatable observations and to deepen the biological, cellular, and molecular basis of these products' mechanism of action (Fleming et al., 2019; Nephali et al., 2020).

Various research approaches have been already proposed over the years to identify specific modes of action of each type of biostimulant. In 2012, Dumas et al. (2012) conducted one of the first studies to understand the changes in gene expression induced by the application of biostimulants, using genetically modified plants. Specifically, the authors observed after application of the biostimulant on *Arabidopsis thaliana* (L.) the stimulation of secondary metabolites involved in the plant's defence mechanisms. In the same year, Gates et al. (2012) suggested that the mode of action of biostimulants can best be determined using molecular microarray analysis to identify gene variations in transcript levels as this approach has the potential to reveal the signalling pathways activated by biostimulants involved in stimulating plant response. In particular, of the 27,400 genes tested on *Arabidopsis thaliana* (L.), nine were identified as significantly up-regulated by the tested biostimulants. These up-regulated genes are involved in the response of plants to various abiotic stresses, including photo-oxidation, cold, salt and drought. However, Summerer et al. (2012) subsequently refuted the hypothesis that the mode of action of biostimulants can be inferred through these procedures due to the need to also integrate phenotyping tests. Indeed, even a complete knowledge of the gene sequence and transcripts in plants cannot reveal the effects of biostimulants on the phenotype. Only the understanding and integration of these information with those of metabolome, which is the final recipient of the flow of biological information (D'Amelia et al., 2018), may give a clear representation of the multilevel effects of biostimulants on plants.

In the following years, Sleighter et al. (2015), Botta et al. (2015), and Conan et al. (2015) focused their studies on the active ingredients contained in biostimulants, with the aim of identifying possible molecular markers. The same authors used proteomic, transcriptomic and metabolomic studies to correlate molecular markers identified with phenotypic observations recorded in plants. However, the complexity of biostimulants, which are composed of more than one active ingredient, makes it difficult to correlate the efficacy of these products with a single component (Gupta et al., 2022). Studies by Bulgari et al. (2019) suggest that the efficacy of biostimulants depends on the synergistic action of the various bioactive components; however, the characterization of these molecules remains inadequate (Nephali et al., 2020). Moreover, many types of biostimulants, such as humic and fulvic acids, plant and animal protein hydrolysates, algae and plant extracts, have a heterogeneous nature that hinders the identification of bioactive molecules involved in their mechanisms of action (Parađiković et al., 2011). The search for the biostimulants' mode of action is further complicated by the fact that many of these products are effective only in plants under suboptimal conditions and/or biotic or abiotic stresses (Yakhin et al., 2017) (Fig. 1). The purpose of this review was to critically analyze the effects of non-microbial biostimulants, of very different nature, on gene expression plasticity, linking molecular mechanisms with phenotypic outcomes, in order to understand their functioning in the plant. In particular, the mechanisms of action of a) protein hydrolysates; b) algae extracts; c) humic acids; and d) silicon will be discussed.

2. Protein hydrolysates

Regardless of the source matrix, protein hydrolysates (PHs) are gaining a significant market share among different types of commercial biostimulants. Their growing use in agriculture can be attributed to their proven effectiveness, but especially because they fit well into a circular economy context, as they are produced from waste generated by industrial and/or agricultural activities (Colantoni et al., 2017; Colla et al., 2017). The remarkable biostimulant action on plant growth and yield is often related to the hormone-like activity of bioactive compounds normally found in PH-based formulations, such as amino acids and signaling peptides, as well as the synergy between other classes of compounds, such as small amounts of carbohydrates, minerals, phenols, and phytohormones (Calvo et al., 2014; Ebinezer et al., 2020). However, it is important to keep in mind that the biostimulant effects are strongly influenced by the raw material (plant and animal) and the hydrolysis process (enzymatic and chemical) used for their production (Colla et al., 2017). Independently of the chemical composition, it is well established that foliar and/or root application of PHs induces targeted primary and secondary metabolic pathways reprogramming in treated plants (Lucini et al., 2015; Sestili et al., 2018) (Table 1). The reviewed literature reported an improvement in RUE due to the action of the dodecapeptide RHPP (root hair promoting peptide), which triggers the remodelling of the root architecture, in particular promoting the expansion and deepening of the root system, thus enlarging the surface of soil-root contact (Colla et al., 2015; Ertani et al., 2013a; Halpern et al., 2015). This has been confirmed by Ceccarelli et al. (2021) who hypothesized that enhanced root development in tomato cuttings treated with hydrolysates of plant-derived proteins (P-PHs) was mainly dependent on higher

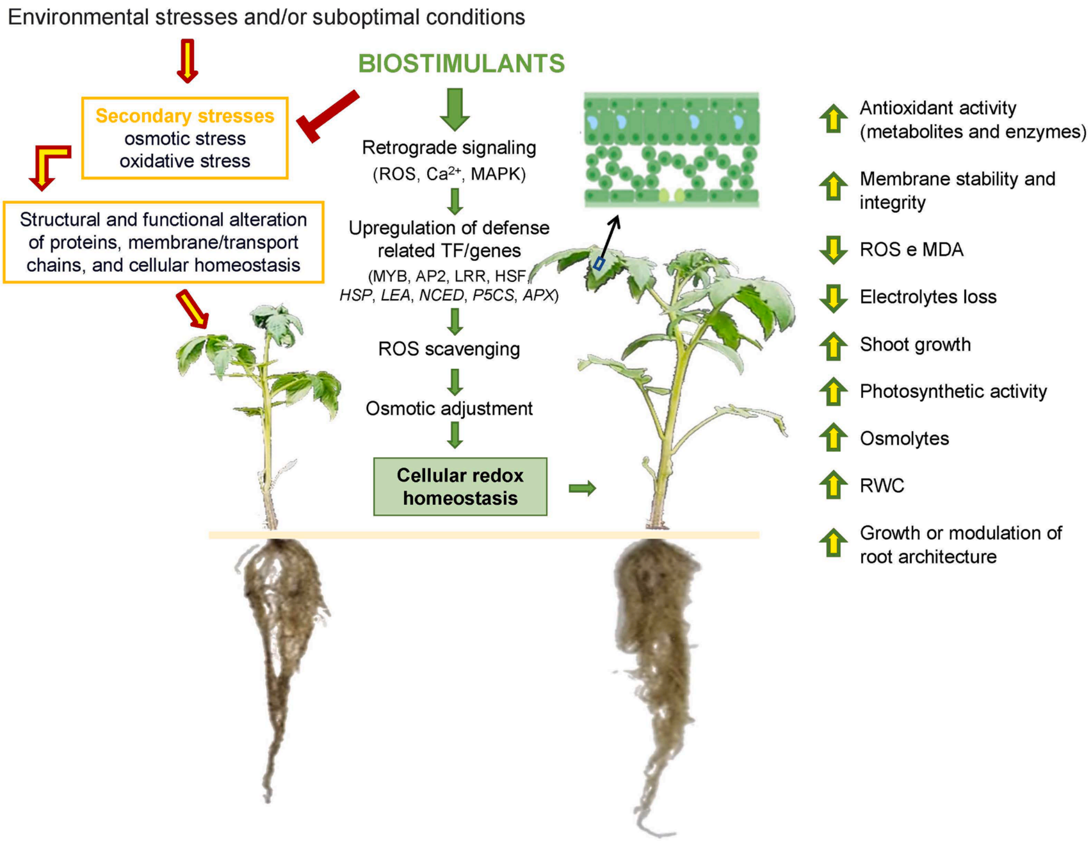

Fig. 1. Environmental stresses and/or sub-optimal conditions commonly cause pleiotropic effects, mainly related to osmotic and oxidative stress. Non-microbial biostimulants play a crucial role in this scenario by inducing the opening of Ca^{2+} channels thus triggering retrograde signaling cascades, including the MAPK and ROS pathways, which transduce cellular dysfunction signals from subcellular compartments to the nucleus. These cause the upregulation of transcription factors (TF) and genes (MYB, AP2, LRR, HSF, *HSP, LEA, NCED, P5CS, APX*) which activate the ROS detoxification system and osmotic adjustment mechanisms, thus restoring cellular redox homeostasis and plant functionality. AP2: APETALA2, APX: Ascorbate peroxidase, LEA: late embryogenesis abundant, LRR: leucine-rich repeat proteins, HSF: Heat shock fctor, HSP: Heat shock protein, MAPK: Mitogen-activated protein kinase, MYB: MYB Proto-Oncogene, NCED: 9-cis-epoxycarotenoid dioxygenase, P5CS: delta1-pyrroline-5-carboxylate synthase.

levels of endogenous indole-3-acetic acid (IAA) precursors such as 4-(indol-3-yl)butanoic acid (IBA) and tryptamine. Furthermore, the authors reported an increase in zeatin (a cytokinin that promotes root elongation) (Guan et al., 2019), a reduction in brassinosteroids (Wei and Li, 2016), and an accumulation of gibberellins (key regulator hormones for root meristem development and organ elongation) (Shtin et al., 2022). The beneficial effects of PHs on the gibberellin signaling pathway have been confirmed by Campobenedetto et al. (2020), who observed a repression of genes involved in the biosynthesis of DELLA proteins (LOC100805968, LOC100791952, GAI and RGL3). Specifically, five different PHs were found to be more effective in promoting root development compared to the exogenous application of IAA in tomato plants (Buffagni et al., 2021). This result was attributed to a greater up-regulation of genes belonging to the auxin/IAA (Aux/IAA) transcription factor (TF) family, specifically *SlIAA2* and *SlIAA9*. However, the same authors highlighted that the five PHs, having a different composition peptides, oligopeptides, and free amino acids, exerted different effects on the modulation of the expression of these two genes (*SlIAA2* and *SlIAA9*). A similar trend was also observed for auxin amino acid conjugates such as MeIAA and IAA-Phe (Buffagni et al., 2021). Transcriptomic and proteomic studies conducted by Xia et al. (2015) revealed that the stimulatory effects of a PH of animal origin (A-PHs) on phytohormones may be caused by specific redox signaling, as there is a well-established relationship between phytohormones and the generation of reactive oxygen species (ROS). The latter, in addition to being the main players in oxidative stress, also function as signaling molecules in plants (Mittler, 2017). However, as reported by Trevisan et al. (2017), modulation of redox homeostasis (i.e., increased glutathione S-transferase, glutathione peroxidase, nucleoredoxins, thioredoxins and dihydrolipoamide dehydrogenase) after application of A-PHs may have regulated various translational and transcriptional mechanisms, contributing to enhance root growth in *Zea mays* L. As supported by Santi et al. (2017), the positive effects of PHs were attributed to the modulation of the main primary metabolic pathways as well as secondary metabolic pathways involved in numerous physiological functions and adaptation to environmental stimuli. Consistent with this, the microarray analysis of Ertani et al. (2017) in tomatoes revealed in root and leaf tissues that the positive effects of a alfalfa P-PH were associated with the up and down regulation of about 32,000 differentially expressed genes. In particular, the increased expression of genes encoding nitrogen metabolism (nitrate reductase, glutamine synthetase, glutamine-dependent asparagine synthetase and aspartate aminotransferase), oxidative and reductive carbon metabolism (fumarate dehydrogenase, malate dehydrogenase, phosphoenolpyruvate carboxylase and phosphoenolpyruvate carboxykinase, RuBisCo), and components of the electron transfer chain (LHCA5 protein and ferredoxin 2). Furthermore, the application of the aforementioned P-PH also modulated the expression of genes encoding enzymes and proteins involved in the uptake and transport of mineral nutrients, particularly nitrogen, phosphorus, potassium, sulphur, iron, and copper. The increase in mineral absorption and translocation in plant tissues is probably attributed to the modification of the root architecture induced by hormone-like signals due to the presence of auxins and amino acid elicitors within the P-PH. Accordingly, Wilson et al. (2018) found that a gelatin, hydrolyzed

Table 1
A summary of the biostimulatory actions of **protein hydrolysate** (PH) applications on molecular mechanisms of plants. AAP3: Amino acid permease 3, SA: salicylic acid, GA: gibberellin, JA: jasmonic acid, PAL1: Phenylalanine Ammonia Lyase 1, PIN1: Auxin efflux carrier component 1.

Type of proteins	Plant species	Biostimulatory mechanisms	References
Legume and leather waste-derived protein hydrolysates	*Solanum lycopersicum* L. cv. Akrai F1	Up-regulation of the key auxin transporter (PIN1).	(Ceccarelli et al., 2021)
Alfalfa-based protein hydrolysate from *Medicago sativa* L. plants	*Solanum lycopersicon* L. cv. Microtom	Upregulation of genes involved in carbon and nitrogen primary metabolism, developmental processes, secondary metabolism and defense system.	(Ertani et al., 2017)
Collagen derived protein thermal hydrolysate	*Zea mays* L.	Remodulation of gene expression in different physiological, metabolic and signalling pathways.	(Trevisan et al., 2017)
Cow connective tissue protein hydrolysate	*Zea mays* L.	Regulation of genes involved in NO$_3^-$ and metal ion transport, C/N ratio signalling in roots, GA and auxin metabolism, signalling and transport.	(Santi et al., 2017)
Different hydrolyzed collagens (mixture of peptides and proteins)	Cucumber, arugula, broccoli, tomato, pepper, corn	Increased expression of root amino acid and nitrogen transporter genes (*AAP3*), enhancing nitrogen uptake.	Wilson et al. (2018)
Lignin and plant-derivates (mixture of amino acids, molybdenum and zinc)	*Glycine* max L. Merr.	Down-regulation of aquaporin genes, up-regulation of genes involved in primary metabolism and stress responses.	(Campobenedetto et al., 2020)
Protein hydrolysate from alfa-alfa (*Medicago sativa* L.)	*Zea mays* L.	Upregulation of a gene involved in salt stress response (*PAL1*).	(Ertani et al., 2013b)
Protein-hydrolysate-based (mixture amino acids and nitrogen)	*Zea mays* L. hybrid Knezha 307	Up-regulation of heat shock proteins in leaves and roots	(Vaseva et al., 2022)

collagen, A-PH biostimulant with high contents of proline and hydroxyproline induced in six crops (arugula, broccoli, cucumber, pepper, tomato and corn) an increased expression of nitrogen and amino acid transporter genes, in particular the *amino acid permease 3* (*AAP3*), involved in long-distance transport of arginine, histidine, and lysine. Regarding the effects on secondary metabolism, Buffagni et al. (2021), Ceccarelli et al. (2021), and Ciriello et al. (2022) reported a significant modulation of nitrogen containing compounds, glucosinolates, alkaloids, terpenes, and phenylpropanoids in response to PH application. As suggested by Ertani et al. (2011), the increased production of phenolic compounds was related to the over-expression of the *phenylalanine ammonia-lyase* (*PAL*) gene, which, as demonstrated by Schiavon et al. (2010) and Ertani et al. (2011), increased upon biostimulant treatment. In a study in lettuce under 40 mM NaCl stress, Rouphael et al. (2022) observed improved productivity when plants were treated with a P-PH, as well as increased biosynthesis of osmoprotectants. Ultimately, the enhanced tolerance to salt stress of PH-treated plants, compared to control ones, was also attributed to an increase in the accumulation of phytoalexin precursors and phenolic compounds, particularly anthocyanins and flavonoids. Similarly, in maize plants, Ertani et al. (2013b) attributed the beneficial effect of a P-PH containing IAA and triacontanol to an increase in proline, flavonoid, and protein content, as well as an efficient re-activation of glutamate synthase (GOGAT) and glutamine synthetase (GS) activities in treated plants. Visconti et al. (2015) observed a reduction in leaf necrosis symptoms in *Diospyros kaki* subjected to salt stress after the application of an A-PH, attributable to reduced chloride uptake and translocation in shoots. Activation of antioxidant enzymes (SOD and APX), coupled with increased biosynthesis of salicylic acid and carotenoids, allowed *Solanum lycopersicum* and *Capsicum annuum* plants treated with P-PHs to improve tolerance to ROS induced by drought (Agliassa et al., 2021; Paul et al., 2019). Lastly, regarding drought stress, Carillo et al. (2019) and (Colla et al., 2015) observed the beneficial effect of P-PHs on the functioning of the photosynthetic machinery in spinach (*Spinacia oleracea* L.) and maize plants, respectively, ascribable to an auxin-like activity of the biostimulant. In maize plants subjected to heat stress, the application of a P-PH provided increased tolerance to drought by upregulating the transcriptional levels of dehydrins and heat shock proteins (HSPs) in leaves (DHNXero1, DHNCor410, DHN2, DHN4, DHN13, HSP16.9, and HSP22) and roots (DHN1 and DHN4, HSP16.9, and HSP116.9), improving protection against protein denaturation and increasing cell membrane fluidity (Vaseva et al., 2022). Ertani et al. (2017) highlighted that an alfa-alfa P-PH might take part in the activation of different protein kinases and TFs involved in complex crosstalk between biotic and abiotic stress signaling pathways thus increasing plant performance. In particular, it induced the expression of multiple TFs involved in the biosynthesis of defence-related proteins, such as the basic helix–loop–helix (bHLH), HSPs, leucine-rich repeat proteins (LRR) and plant aldo-keto reductases (AKR), to name a few. Trevisan et al. (2017) evidenced that also a collagen derived A-PH could induce in roots and shoots of maize a differential expression of TFs families such as the ethylene-responsive factors (ERF), the basic leucine zipper (bZIP) family, the WRKY TFs (name derived from the highly conserved 60 amino acid long WRKY domains containing at the N-terminus the conserved heptapeptide motif WRKYGQK), and myeloblastosis protein (MYB) family (TRASPARENT TESTA). Accordingly, Santi et al. (2017) showed that an A-PH could induce a different transcriptional profile, which not only involved the regulation of the biological and cellular metabolic processes previously described, but also the expression of TF families belonging to the APETALA2 (AP2)/Ethylene Responsive Element Binding Factor (EREB) domain, bHLH, WRKY, MYB, in addition to the NAC (NAM, ATAF and CUC) TF senescence network. Therefore, PH-induced TFs modulation may represent an alternative to genetic engineering to enhance abiotic stress tolerance.

3. Seaweed extracts

Seaweed extracts (SWEs) are rapidly gaining a significant share of the market, accounting for more than 33 % of biostimulant products (Bio4Safe, 2018). Recent surveys estimate that the seaweed market will experience significant growth between 2023 and 2030, with a CAGR of 12.9 %, exceeding $2580 million (GlobalNewsWire, 2023). Algal products are not limited to agriculture, as historical studies have highlighted humans have used them as a food source, folk medicine, cosmetics, textiles, and dyes (Battacharyya et al., 2015). Within the agricultural field, the use of seaweed is not a novelty. In various geographical areas, from Great Britain to Japan, algal biomass has been widely applied to the soil as a fertilizer or soil amendment to improve its physicochemical properties (Carillo et al., 2020). The intensification of sustainable agricultural practices has focused attention on the biostimulant activity of SWEs, as they are capable of improving the productivity and quality of horticultural crops, as well as the response to abiotic stresses, while increasing the efficiency of use of non-renewable resources that must be preserved for future generations (Colla and Rouphael, 2015; Deolu-Ajayi et al., 2022; Sangha et al., 2014). (Table 2).

Algae-based biostimulants do not represent a homogeneous category of products (Carmody et al., 2020). It is well recognized that the type, source, and season of raw materials harvesting, together with the extraction method used, contribute to the significant heterogeneity in the chemical composition of commercial SWE (Craigie, 2011). This variation in chemical composition accounts for the diverse range of biological effects observed in SWE treated plants. The positive effects on plant productivity and resilience resulting from the use of SWE as a fertilizer can be attributed to two key factors: the enhancement of soil structure and the provision of essential nutrients found in seaweeds. However, thorough investigations of the mineral composition of biostimulants derived from SWEs have revealed that the relatively low levels of macronutrients present cannot fully explain the observed physiological responses (Battacharyya et al., 2015). To address any uncertainties, it is crucial to remember that these products are generally applied weekly and in small quantities (Carillo et al., 2020). For this reason, the biostimulant activity of SWEs can be attributed to the activation of specific signaling pathways induced by a wide variety of metabolites such as hormone-like compounds, carbohydrates, vitamins, polyamines, carotenoids, amino acids, betaines, and phenols (Battacharyya et al., 2015; Carillo et al., 2020; Sujeeth et al., 2022). Studies have provided evidence to support the positive impact of algae extracts on root structure and nutrients uptake. Specifically, SWE polysaccharides, such as alginates and fucoidans,are able to create a polymeric networks improving soil structure and aeration, and promoting root growth (Luthje and Bottger, 1995) and soil microbiome thus improving the water retention capacity and facilitating the chelation of metal ions (Anderson, 2009; Craigie, 2011; Hegazy et al., 2009; Lattner et al., 2003; Verkleij, 1992). Furthermore, beneficial effects can also be attributed to the activity of a derivative of vitamin K1 known as kahydrin that, by modifying the proton pumps in the plasma membrane, stimulates the secretion of protons into the apoplast leading to acidification of the rhizosphere. This acidification process facilitates the cation–anion exchange in the soil increasing the availability of nutrients for the uptake of the plant (Luthje and Bottger, 1995). Therefore, plant growth can be attributed to the effects of algae extracts on the regulation of genes involved in the primary active transport and/or uptake of inorganic compounds, particularly minerals (Shukla et al., 2019). Through microarray analyses of gene expression profiling, researchers have observed a distinct regulation of 729 and 298 genes in shoots and roots, respectively, of *Brassica napus* treated with *Ascophyllum nodosum* extracts, even after just three days of treatment (Jannin et al., 2013). Algae extracts were found to upregulate genes responsible for sulphate assimilation (*BnSultr1.1* and *BnSultr1.2*), nitrate assimilation and amino acid metabolism (*BnNRT1.1* and *BnNRT1.2*), in addition to auxin transport (Castaings et al., 2011; Krouk et al., 2010). Other studies have also reported the effects on gene regulation exerted by *A. nodosum* extracts. For example, these SWE caused the overexpression of the *COPT2* and *NRAMP3* genes that regulate the translocation of copper, iron, and zinc, respectively (Billard et al., 2014). Similarly, another study by Goñi et al. (2021) reported the increased expression of various genes involved in mineral transport, including *NRT1.5* (nitrate), *CAX3, CAX7* and *ACA1* (calcium), *COPT2* (copper), as well as *SULTR1, SULTR3* and *AST56* (sulphate). Furthermore, the study found an up-regulation of genes related to amino acid transport, such as *LHT1* and *AAP5*, peptide transporters such as *ATOPT3*, nucleotide sugar derivatives (*UTR2* and *UTR3*), nucleotides (*ATPUP10*) and sugars (*MSS1*).

The phenotypic changes observed in plants treated with SWE can also be attributed to the modulation of biosynthesis and the balance of endogenous hormones, including auxins, abscisic acid (ABA), cytokinins and gibberellins (Sujeeth et al., 2022). However, it is worth noting that these responses may be not directly related to the presence of phytohormones in the SWE itself, but rather to unique chemical components

Table 2

A summary of the biostimulatory actions of **algal extracts** application on molecular mechanisms of plants. ABA: abscisic acid, Annexin: ANN, AP2: Apetala 2, APX: Ascorbate peroxidase, CK: cytokinins, CHS: Chalcone synthase, CYCP2.1: cyclin-like protein, GPX: Glutathione peroxidase, HSP: Heat shock protein, LEA: Late embryogenesis abundant; LTPs: Lipid-transfer proteins, MAP: Mitogen-activated protein, MYB: MYB Proto-Oncogene, NCED3: 9-cis-poxycarotenoid dioxygenase 3, NHX: Na+, $K+/H+$ antiporters, NRT: Nitrate transporter, PIP1: Plasma Membrane Intrinsic Protein1, P5CS: delta1-pyrroline-5-carboxylate synthase, SnRK2: SNF1-related protein kinases, SOS1: Salt Overly Sensitive 1, WAK1: Wall-associated kinase 1, WRKY: transcription factors containing the WRKY domain.

Algae species	Plant species	Biostimulatory mechanisms	References
Ascophyllum nodosum	*Arabidopsis thaliana*	Regulation of genes involved in ion transport and transmembrane proteins aided by ATPases.	(Jithesh et al., 2012a)
Ascophyllum nodosum	*Arabidopsis thaliana* and winter barley plants (cv. Towers)	Increase the expression of three nitrate plasma membrane carriers (NRT1.1, NRT1.5, and NRT2.1).	(Goñi et al., 2016; Jithesh et al., 2019b)
Ascophyllum nodosum	*Arabidopsis thaliana*	Up-regulation of genes involved in stress responses (*LEA*) and in lipid degradation; down-regulation of genes associated with fatty acid and phospholipid synthesis.	(Goñi et al., 2016; Jithesh et al., 2019b)
Ascophyllum nodosum	*Arabidopsis thaliana*	Up-regulation of genes involved in stress responses (*LEA*), carbohydrate metabolism, lipid transport (LTPs) and transcription factor (zing-finger, MYB and AP2). Down-regulation of kinase (WAK1), pectin-esterases and transcripts of RNA-binding proteins.	(Jithesh et al., 2019b)
Ascophyllum nodosum L. Heyhn.	*Arabidopsis thaliana*	Upregulation of CK and ABA biosynthetic genes.	(Wally et al., 2013b)
Ascophyllum nodosum	*Asparagus aethiopicus* L.	Increased expression of genes responsible for water management (*ANN1, ANN2* and *PIP1*), the production of proline (*P5CS1*) and secondary metabolites (*CHS*) and the accumulation of antioxidants (APX1 and GPX3) to combat salt stress.	(Al-Ghamdi and Elansary, 2018a)
Lessonia nigrescens	*Triticum aestivum* L. Jimai 22	Overexpression of genes responsible for the compartmentalization of Na+, thus protecting the plant (*NHX2* and *SOS1*)	(Zou et al., 2019b)
Ascophyllum nodosum	*Arabidopsis thaliana*	Up-regulation of gene responsible of ABA biosynthesis (*NCED3*).	(Santaniello et al., 2017)
Gracilaria dura	*Triticum aestivum* L.	Enhanced expression of genes responsible for ABA biosynthesis against drought stress.	(Sharma et al., 2019)
Ascophyllum nodosum	*Lycopersicon esculentum*, cv. MicroTom	Upregulation of heat shock proteins (HSP101 and HSP70.9).	(Carmody et al., 2020)
Ascophyllum nodosum	*Arabidopsis thaliana*	increased expression of genes involved in proline synthesis (*P5CS1* and *P5CS2*) against low temperatures.	(Nair et al., 2012)
Seaweed-Based extract	*Arabidopsis thaliana*	Up-regulation of genes responsible for photosynthesis, hormone signalling and growth-	(Sujeeth et al., 2022)

(*continued on next page*)

Table 2 (*continued*)

Algae species	Plant species	Biostimulatory mechanisms	References
		related genes under oxidative stress.	
Ascophyllum nodosum	*Arabidopsis thaliana*	Increased expression of growth-promoting genes (*CYCP2;1*) during drought stress.	(Rasul et al., 2021; Staykov et al., 2020)

capable of triggering endogenous hormonal-like signaling, thus regulating plant growth (Sujeeth et al., 2022). For example, studies using UPLC-ESI-MS/MS by Wally et al. (2013a) revealed that the concentration of phytohormones in various algae-based biostimulant products was insufficient to account for the observed effects on yield, given the low dosage typically used. Furthermore, the application of SWEs was found to regulate the transcription levels of specific genes involved in cytokinin biosynthesis (such as *IPT3, IPT4* and *IPT5*), leading to increased cytokinin production through the mevalonate pathway and suppression of genes responsible for cytokinin degradation. However, the improvement in vegetative growth and reduced root length were attributable to the increase in ABA and the reduction in IAA levels (Wally et al., 2013a). In another study by Rayorath et al. (2008), the application of the *A. nodosum* extract, even without detectable levels of gibberellic acid, activated the expression of α-amylase genes in barley, suggesting the presence of other bioactive compounds in the extract responsible for the enhancement of breakdown of starch reserves for seed germination. Furthermore, it is important to note, as suggested by Sujeeth et al. (2022), that endogenous hormonal regulation induced by the application of algae extracts is pivotal in helping plants coping with various stressors.

Biostimulants provide the greatest benefits to plants when they are subjected to stressful conditions. In this sense, the application of SWEs plays a crucial role in improving RUE and promoting the synthesis of protective compounds. These extracts activate the defence mechanisms of the plant, safeguarding its cellular and subcellular structures. Numerous scientific studies have shown up-regulation of various TFs (such as the DRE binding protein, Dehydrin COR47, COR15A, NFYA, LHY1, CCA1 and AGF2) involved in stress tolerance, both at transcriptional and post-transcriptional levels (Goni et al., 2016; Jithesh et al., 2019a; Shukla et al., 2019).

When plants are grown in environments with high salinity levels, the application of SWEs can trigger defensive mechanisms that improve salt tolerance. These mechanisms include the overexpression of late embryogenesis abundant (LEA) proteins, which prevent proteins aggregation under water-deficit conditions, as well as ion sequestration, and improve water status (Goyal et al., 2005; Jithesh et al., 2019a; Wise and Tunnacliffe, 2004). For example, a study on asparagus reported an increase in the expression of genes (such as *PIP1, ANN1*, and *ANN2*) involved in the response of the plant to salt stress after the application of an extract of *A. nodosum* (Al-Ghamdi and Elansary, 2018b). However, the authors also reported an up-regulation of the genes *chalcone synthase* (*CHS*) and *delta1-pyrroline-5-carboxylate synthase* (*P5CS1*) responsible for the synthesis of chalcone and proline, which are involved in the biosynthesis of secondary antioxidant metabolites (phenolic acids, flavonoids, and isoflavonoids) and in balancing osmotic pressure and stabilization of the protein membrane (Dao et al., 2011), respectively. Studies in turfgrass (Elansary et al., 2017) and *Arabidopsis* (Jithesh et al., 2019a) demonstrated an improvement in salt tolerance attributed to the reduction of oxidative damage mediated by specific enzymes and glutathione S-transferase. Furthermore, the use of SWEs has been found to improve the efficiency of phosphorus and Sulphur use (Shukla et al., 2019, 2018). This effect is achieved through positive modulation of miRNAs (such as *miRNA395, miRNA398, miRNA399, miRNA827* and *miRNA221b*), which are involved in the regulation of plant responses to oxidative stress, water shortage, and ABA (Shukla et al., 2018; Zhu et al., 2011). In the case of wheat, the polysaccharide component of the *Lessonia nigrescens* extract has been credited with mitigating the oxidative damage caused by salt stress. This component induces overexpression of genes (such as *NHX2* and *SOS1*) responsible for the compartmentalization of Na^+, thus protecting the plant (Zou et al., 2019a). Jithesh et al. (2012b), on the other hand, observed that extracts based on *A. nodosum* simultaneously upregulated the genes responsible for dehydrin synthesis and downregulated the negative regulators of salt stress. Recently, it has been reported that dehydrins not only function as chaperone, chelator and cryo-protectant stabilizing biomolecules and membranes, but they may regulate stress-responsive genes, participating in the cell's transcription regulatory machinery and/or in epigenetic modification during the stress response (Tiwari and Chakrabarty, 2021).

Similar to the effects observed in salt stress, the relief effects of SWEs on water stress can be attributed to specific bioactive compounds that regulate and modulate antioxidant, molecular, and physiological responses. In particular, Santaniello et al. (2017) reported an up-regulation of the *AtNCED3* gene, responsible for ABA biosynthesis, in plants subjected to water stress and treated with SWEs. This up-regulation resulted in improved water use efficiency (WUE) by regulating stomatal closure. Similarly, under drought conditions, the application of the *Gracillaria dura* extract modulated ABA homeostasis in bread wheat through the expression of *9-cis-epoxycarotenoid dioxygenase* (*NCED*) genes, such as Ta*NCED3.1* and Ta*NCED3.2* (Sharma et al., 2019). However, the positive impact of SWEs on plants experiencing water stress extends beyond stomatal regulation, as they also down-regulate the expression of genes involved in RuBisCO activation (such as *AtRBCS1A* and *AtRCA*) and protection of photosystem II (*GmFIB1a*) (Demirevska et al., 2008; Santaniello et al., 2017; Shukla et al., 2018). Furthermore, SWEs exhibit potent ROS scavenging activity, activating both enzyme and non-enzymatic antioxidants (Elansary et al., 2017). To support this, Tinte et al. (2022) demonstrated a reprogramming of the phenylpropanoid biosynthetic pathway in water-stressed maize plants treated with SWEs. This pathway plays a crucial role in the production of specialized metabolites involved in plant defense. The study revealed an increase in the production of caffeic acid, ferulic acid, coumaroylquinic acid, caffeoylquinic acid, coumaric acid, phenylalanine, and coumaraldehyde. In particular, coumaraldehyde is particularly important as it contributes to strengthening the cell wall in stressed plants by serving as a precursor in lignin biosynthesis (Barba et al., 2015; Stirk et al., 2013).

The application of *A. nodosum* SWEs had a positive effect specifically on tomatoes exposed to high temperature heat stress. In particular, their application maintained optimal levels of nonphotochemical quenching yield (ΦNPQ) and preserved pollen viability compared to stressed plants not treated with SWEs (Carmody et al., 2020). The authors suggested that this result could be attributed to a higher content of simple sugars (glucose, fructose, and sucrose) compared to stressed plants not treated with SWE. Furthermore, a SWE increased the expression of HSP genes in anthers and young fruits, which are responsible for the activation of HSPs, such as HSP101.1 and HSP70.9, that play a key role in heat tolerance (Carmody et al., 2020). Similarly to what has been mentioned about high temperature stress, Rayirath et al. (2009) and Nair et al. (2012) observed that the lipophilic fraction of the SWE improved tolerance to freezing in *Arabidopsis thaliana*. Rayirath et al. (2009) observed a reduction in electrolyte leakage in plants treated with a SWE compared to untreated frozen plants, indicating the maintenance of cell membrane integrity during exposure to low temperatures. The same authors also highlighted an up-regulation of genes that respond to low temperatures (*COR15A, RD29A* and *CBF3*) and modulation of chlorophyllase gene expression (*AtCHL1* and *AtCHL2*). Furthermore, Nair et al. (2012) explored the putative mechanisms underlying the improved tolerance of plants subjected to cold stress and treated with SWEs. They found that the application of the lipophilic fraction of the *A. nodosum* extract to plants exposed to a temperature of −2 °C altered the expression of more than 1000 genes encoding the accumulation of sugars, lipid metabolism products, and osmoprotectants such as proline. Specifically, the authors observed an increased expression of genes involved in the synthesis of proline synthesis (*P5CS1* and *P5CS2*), while the *proline dehydrogenase* gene (*ProDH*) decreased. Nair et al. (2012) concluded that the enhanced tolerance of plants to cold stress could be attributed to the secondary metabolite profile of the lipid fraction of the SWE, which regulated the levels of soluble sugars, sugar alcohols, organic acids, and important membrane and organelle stabilisers such as fatty acids.

The effectiveness of SWEs applied as a leaf pretreatment was evaluated in a recent study by Sujeeth et al. (2022). The authors pretreated *A. thaliana* seedlings with a SWE and subsequently sprayed them with N, N'-dimethyl-4,4′-bipyridinium dichloride (trade name Paraquat), a non-selective desiccant herbicide known to induce ROS production and oxidative stress. The results of their study revealed that leaf pretreatments with the SWE protected plants from herbicide-induced oxidative damage. These findings were supported by molecular, transcriptomic, physiological, and lipidomic analyses. Specifically, pretreated plants did not showed no leaf damages, absence of autophagy and programmed cell death gene expression, reduced expression of ROS marker genes, increased accumulation of organic acids, maltose and raffinose, and decreased levels of triacylglycerol (TAG). The beneficial effects of leaf pretreatments with SWEs were also confirmed in pepper and tomato plants (Staykov et al., 2020), as well as in another study on *A. thaliana* (Rasul et al., 2021) under drought stress, where SWEs reduced hydrogen peroxide (H_2O_2) accumulation, induction of ROS marker genes, and maintained the expression of the *HIS4* gene (a cell cycle marker gene). The authors emphasize that the beneficial effects of leaf pretreatments were also associated with the enhancement of metabolic pathways that lead to the formation of cytokinin 2 signaling compounds (ARR2), apoplastic peroxidase (PRX34) and ABA-dependent genes (*RCAR3* and *RBOHD*).

Table 3

A summary of the biostimulatory actions of **humic substances** application on molecular mechanisms of plants. ABA: abscisic acid, AP2: Apetala 2, CSD: copper/zinc superoxide dismutase, CYP: cytochrome P450, EIN2: Ethylene insensitive 2 protein, GRF: Growth-regulating factor, HSP: Heat shock protein, LAC: Laccase, LOX: Lysyl Oxidase, MAPK: Mitogen-activated protein kinase, MYB: MYB Proto-Oncogene, NAC: NAM/ATAF1/2/CUC2 transcription factor, NAM: PM- H^+-ATPase: plasma membrane proton pumping, H^+-ATPase TF: Transcription factor, WRKY: transcription factors containing the WRKY domain.

Type of humic acid	Plant species	Biostimulatory mechanisms	References
HA (Sigma-Aldrich)	*Arabidopsis*	Transcriptional expression of HSPs	Cha et al. (2020)
Coal-based humic acid (CHA), fulvic acid (FA), biochemical humic acid (BHA),	*Cucurbita pepo* L.	Improved cold stress tolerance by plant metabolism reprogramming.	Li et al. (2023)
Humic substances (HS)	*Pelargonium* × *hortorum* L. *H. Bailey 'Freckles' and Tagetes patula* L. *'Bonanza'*	Increased fresh and dry weight, length and number of roots at low HS concentrations of 50–100 mg/L.	Hartwigsen and Evans (2000)
Sedimentary humic substances	*Cucumis sativus* L.	Root ABA increase correlated to enhancement of root hydraulic conductivity and shoot growth.	Olaetxea et al. (2015)
Humic acids from vermicompost	*Zea mays* L. *DKB 789*	Enhanced expression of glutathione and peroxidase proteins, higher root diameter, length, number and fresh weight correlated with an increase in antioxidant responses.	Nunes et al. (2019)
Humic substances	*Triticum aestivum* L.	Up and down regulation of *AP2, GRF, LAC, CSD1/CSD2*, and plastocyanin genes.	Arsanal et al. (2021)
Earthworm compost	*Zea mays* L., var. Dekalb 177	Modulation of cell receptors, phosphatases, MAPK and metabolic hormones pathways.	Souza et al. (2022)
Humic acids isolated from vermicompost in combination with *Herbaspirillum seropedicae* strain HRC54	*Zea mays* L. var. UENF 506–11	Upregulation of expression of PM-H^+-ATPase and aquaporins, and repression of nitrate transporters.	de Azevo et al. (2022)
Leonardite coal mines	*Lilium longiflorum* L. cv. Dynamix	Regulation of gene involved in senescence process (*CYP, NAC2, LOX, EIN2*).	Rahbar et al. (2022)
Coal-based humic acid (CHA)	*Cucurbita pepo* L. cv. SH-3	Modulation of calmodulin and phytohormone signalling, glutathione metabolism and peroxidase synthesis. Regulation of TFs (NAC, WRKY and MYB genes) involved in stress tolerance.	Li et al. (2023)

4. Humic acids

Humic substances (HS) derive from the chemical and/or biological decomposition of organic waste and are natural constituents, up to 80 %, of the organic fraction of the soil; therefore, they are considered the most abundant organic matter on earth (Calvo et al., 2014; du Jardin, 2015). HS are heterogeneous polymeric compounds very resistant to degradation, which do not have a specific chemical formula (e.g., they can be partially aromatic, acid, colloidal and hydrophilic) or molecular weight (Canellas et al., 2015). However, based on their degree of solubility, they are classified in i) humins, not soluble in water and not extractable from the soil, ii) humic acids (HA), soluble in water at basic pH, and iii) fulvic acids (FA), soluble in water at any pH (Bulgari et al., 2015). Their presence influences the physicochemical properties of the soil by changing its structure and texture; in fact, they increase the soil matric potential (SMP) and enhance the amount of water held in the soil profile for plant uptake/use (Table 3). Moreover, they are able to buffer the pH of the soil (du Jardin, 2015) and create soluble complexes with micronutrients (e.g., iron), positively affecting the soil cation exchange capacity, counteracting the nutrient leaching and enhancing nutrients availability to plants (Ampong et al., 2022). High molecular weight HS may enter into the apoplast and interact only with plant cell walls, while low molecular weight FA that can cross the cell wall and enter in contact with the plasma membrane. at the interface of which they release the micronutrients they are complexed with (Trevisan et al., 2010). In fact, thank to their amphiphilic nature, HA and FA can chelate and co-transport beneficial micronutrients to plant apoplast, while reducing and precipitating toxic heavy metals (HMs) thus reducing their intake by plant (Wu et al., 2017; Yang et al., 2021). The carboxylic, hydroxyl and phenolic groups of HS, in fact, are able to complex with macronutrients (phosphorus) and metallic ions (iron, copper, manganese, and zinc) increasing their availability to plants (García et al., 2016). Moreover, HS chemical moieties are the major binding sites for HMs and herbicides present in solution, to which they also can be complexed (e.g. free lead ions), thus reducing their uptake and translocation in plant tissues, and preventing and/or limiting their entry into the food chain (Santos et al., 2014). In particular, HS' photogenerated hydroxyl radicals may enhance the photolysis and the detoxification of herbicides and pesticides (Zeng at al., 2002), but only at specific HS concentration ranges (30 mg L^{-1} for atrazine and 10 mg L^{-1} for iprodione); while at higher concentrations, they reduce their photolysis and degradation (Garbin et al., 2007). HS were also supposed to play direct actions by targeted and non-targeted effects at plant cell membranes of roots and shoot inducing transcriptional and post-transcriptional modifications (Van Oosten et al., 2017). However, only recently the molecular mechanisms responsible for the HS effects on plants have begun to be elucidated (Ampong et al., 2022). Cha et al. (2020) performed a genome-wide transcriptomic study after suppling HS with MS medium to Arabidopsis plants under heat stress. Results showed 3257 genes differently regulated by HS, 1677 of which up-regulated and involved in different biological processes including heat-stress tolerance by inducing the expression of HSP100 and HSP90 gene families. On the contrary, plants of Arabidopsis unable to express the protein HSP101 essential for the heat stress tolerance (HPS101 null mutant, *hot1*), were insensitive to HS and prone to heat stress, suggesting that HSP101 is a molecular target of HS to induce heat-stress tolerance in Arabidopsis (Cha et al., 2020). HSPs play an important role as molecular chaperones avoiding that cell proteins undergo thermal denaturation or unfolding and facilitating refolding of denatured substrates. However, HSPs/chaperones are involved not only in the response to heat stress but also to drought, salt, osmotic, high light, cold, oxidative stress, UV and pathogens (Swindell et al., 2007). Their 'client proteins' may be enzymes of primary metabolism or transcription factors with pivotal roles for plant cell functioning (Jacob et al., 2017).

Li et al. (2023) investigated the effects of three HS, coal-based humic acid (CHA), fulvic acid (FA), and biochemical humic acid (BHA), on the expression of genes coding for calmodulin, peroxidases, ethylene-responsive TFs, and NAC and WRKY TFs, in leaves of zucchini seedlings under cold stress. The transcriptional analysis evidenced that HA improved cold stress tolerance by reprogramming plant metabolism and modulating stress responses and signal transduction pathways.

Hartwigsen and Evans (2000) showed first that HS may enhance rhizogenesis in geranium and marigold, increasing the length and number of roots when supplied at low concentrations (50–100 mg/L), thus increasing also the root and shoots' fresh and dry weight. Olaetxea et al. (2015)Olaetxea et al. (2015) have suggested that the interaction between sedimentary HS and roots, with partial reduction of root hydraulic conductivity, because of a fouling effect, may induce a transient mild water stress (water eustress) able to promote a gene-mediated stress resistance response. Clearly, if the concentration of HS is too high, the stress effect cannot be overcome. In fact, *Cucumis sativus* plants treated with low levels of sedimentary HS showed a significant increase of concentration of ABA in roots associated with an enhancement of root hydraulic conductivity and shoot growth (Olaetxea et al., 2015). HS increased also root and shoot growth, leaf hydration and antioxidant response in *Agrostis palustris* and *Festuca arundinacea* under drought stress (Shah et al., 2018). In a proteomic study, Nunes et al. (2019) determined in maize roots treated with HA (50 mg CL^{-1}) higher root diameter, length, number and fresh weight than untreated control. These beneficial effects were correlated with an enhanced expression of glutathione and peroxidase proteins involved in antioxidant responses (Nunes et al., 2019).

Zandonadi et al. (2010) proved that the HA-induced lateral root formation is a nitric oxide (NO)-mediated process. The changes in root growth and morphology induced by HS have initially been correlated to the action of phytohormones, in particular IAA, contained in HS (Muscolo et al., 1998). However, subsequent studies proved that purified HS, without measurable concentrations of any plant hormone, could induce in maize and cucumber the root tissue accumulation of nitric oxide (NO), in addition to IAA and ethylene (Mora et al., 2012; Zandonadi et al., 2010). Therefore, molecules and/or molecular domains with hormone like activity present in HS may be responsible for the induction of phytohormones synthesis and root growth effects. In particular, the increase of plasma membrane (PM) H^+-ATPase activity responsible for the maintenance of electrochemical gradient, active primary transport and cell wall relaxation during root cells elongation, may by modulated by biochemical pathways involving HS-induced NO (Zandonadi et al., 2010). Accordingly, de Azevedo et al. (2019) found in roots of maize seedlings that HA upregulated the genes PM-H^+-ATPase (*Mh1*), aquaporin 1 (*PIP1*) and nitrate transporters (*Nrt2.1* and Nrt1.1).

Zandonadi et al. (2019) identified in the unbound fraction of HS obtained from cattle manure vermicompost an alkamide (e.g., N-isopropyldecanamide). Alkamides are affinin growth regulators able to induce auxin-independent growth of lateral roots and elongation of hair roots, in addition to transcriptional re-programming and metabolic modulation (Campos-García et al., 2021). In fact, HS application determines changes in plant secondary metabolism, promoting the accumulation of antioxidant compounds and enzymes (Colla et al., 2015). A microarray study of Jannin et al. (2012) on winter oilseed rape coupled with physiological, biochemical and light and electron microscopic analyses, demonstrated that HS enhanced the root nitrate uptake, up-regulated the expression of the root nitrate transporter *BnNRT2.1* and those of the sulfur transporters *BnSultr1.1* and *BnSultr1.2*, thus improving the N and S transport to shoot with a fast increase of the number of chloroplasts per cell, photosynthetic activity and starch granules accumulation per cell. FA, specifically, have been found to upregulate the transcription of genes related to nutrient transporters and N metabolism in the roots of *Medicago sativa* (Capstaff et al., 2020). Using next generation sequencing (NGS), Priya et al. (2014) found that FA could upregulate the expression of genes involved in K transport and *mobilization* activating the auxin-signalling pathway. Since auxin usually plays a role of signal for low K, inducing the plant to upregulate the expression of high affinity transporters and/or channels for K uptake and transport, the auxin-like activity of FA may exert the same effect independently of the presence of the phytohormone. Moreover, FA increased the expression of genes coding for enzymes of sugar metabolism, (e.g., glucan water dikinase, amylase and isoamylase, involved in stored starch degradation) (Priya et al., 2014). Sun et al. (2020) performed a transcriptomics and metabolomics profiling in tea plants under drought stress and FA treatment. The genes (3331) and metabolites (125) differently expressed under 8 days drought in FA treated plants belonged to ascorbate, glutathione, and flavonoids pathways. This FA-dependent upregulation of antioxidant enzymes and metabolites allows tea plants to improve the drought-stress resistance by increasing the scavenging mechanisms and antioxidant systems (Sun et al., 2020).

Arslan et al. (2021) studied the effect of HA on the expression of miRNA drought-related target genes (*AP2, GRF, LAC, CSD1/CSD2*) and plastocyanin in tolerant and susceptible wheat genotypes trying to unravel the ABA dependent or independent pathways involved in the response to 6 bar and 8 bar PEG6000 stress treatments. The expression analysis showed that all genes were down or up-regulated, even if the genes expression changed depending on plant tissues and genotypes. In particular AP2 promoter was downregulated by HA through an ABA-independent pathway since it did not show boxes related to ABA. The AP2 TF NtERF172 binds to the NtCAT promoter and plays an important role in maintaining the homeostasis of H_2O_2 under drought stress (Zhao et al., 2020); its downregulation prevented the scavenging of H_2O_2, thus amplifying the H_2O_2 stress signalling cascade. Overall, the results of Arslan et al. (2021) showed that HA and stress application induced the upregulation of growth-regulating gene factors (GRF) in leaves of tolerant wheat genotypes compared to sensitive ones. The promoter analysis also showed that HA supply caused the upregulation in the root tissues of sensitive and tolerant genotypes of the ABA-related ABRE motif positively modulating, by the activation of NO-IAA signal pathway, the multigene family coding for laccases. These latter are copper-containing polyphenol oxidases oxidizing several aromatic and non-aromatic compounds in presence of oxygen, among which monolignols to produce lignin, pivotal for plant growth and stress responses (Bai et al., 2023).

Souza et al. (2022) studied the key role of HA in activating different metabolic pathways in maize root seedlings involving the crosstalk of plant hormones, the receptors activation, and the phosphatases activity. Among the different metabolic pathways analysed, that involving auxin showed the main changes regarding the overexpressed genes compared to those entailing ethylene, ABA and JA. Moreover, the transcription analysis showed that HA was able to activate several receptors and different classes of phosphatases genes, further proving the role of HA as a key player in the coordination and interconnection of different hormonal metabolic pathways boosting plant stress responses (Souza et al., 2022). HA can not only influence plant hormones crosstalk but has also a hormone-like activity as proved by the study of Rahbar et al. (2022) on antioxidant defence system and senescence-related genes expression in petals of a *Lilium longiflorum* hybrid. In particular they studied the changes in transcript levels of genes coding for cytochrome P450 monooxygenase (CYP), lipoxygenase (LOX) and NAC2 TF potentially involved in petal development and senescence and prodromic for JA synthesis. HA through an auxin-like effect reduced the expression of CYP and LOX while increased that of NAC2 delaying senescence and durability of petals. Also NAC family appeared involved in flowering senescence (Rahbar et al., 2022).

5. Silicon

Silicon (Si) containing organic components such as oxygen, hydrogen, and carbon, but considered an inorganic or hybrid substance, is well known in the electronic industry, and has recently gained attention in agriculture both as pests and diseases control agent, and biostimulant (Hidalgo-Santiago et al., 2021). In fact, notwithstanding Si is not considered an essential nutrient for most plants, it is actively accumulated by plants enhancing their growth and improving tolerance to various biotic and abiotic stresses (Liang et al., 2015c). Si can upregulate genes in rice involved in its own uptake and trasport, and, in particular, for those coding for the plasma membrane influx transporter LSI1 responsible for Si uptake into the exodermis, the efflux transporter Lsi2 for extruding Si into the apoplast across the aerenchyma; and the trasporter Lsi6 moving Si to the aerial parts of the plant, respectively (Kaur and Greger, 2019; Ma et al., 2011) (Table 4). Si, present in the soil soution as H_4SiO_4, may be easily uptaken by roots and translocated to the areal parts of plants where it accumulates (Hodson et al., 2005). However, its efficacy is still debated due to different effects it exerts on different species and/or genotypes under different environmental conditions (Wang et al., 2021). Si-dependent water stress tolerance has been related to the ability of Si to help preventing water loss through transpiration (Rafi et al., 2020). However, the way in which Si ameliorates plant water status seems more depending on its ability to increase root water uptake and retention capacity, rather than decreasing water loss by osmotic adjustment (Chen et al., 2018). In *Sorghum bicolor*, Si supply upregulates the expression of *SbPIP1;6, SbPIP2;2*, and *SbPIP2;6* genes thus improving the synthesis and activity of the plasma membrane intrinsic protein (PIP), the main root aquaporins pivotal for uptaking and moving water molecules across cell membranes, in particular under abiotic stresses (Liu et al., 2014).

Table 4
A summary of the biostimulatory actions of **silicon** application on molecular mechanisms of plants. CSD: Copper/zinc superoxide dismutase, DREB2A: Dehydration-responsive element-binding protein 2A, Lsi1: Aquaporin-like transmembrane protein and root-specific silicon transporter, Lsi2: Anion transporter superfamily and efflux transporter of silicon, NAC5: NAC family transcription factor 5, POD: Peroxidase, SOD: Superoxide dismutase.

Silicon	Plant species	Biostimulatory mechanisms	References
/	*Sorghum bicolor* L.	Up-regulation of genes coding for plasma membrane aquaporins under water deficit stress	Liu et al. (2014)
/	*Oryza sativa* L. cv Fajr	Overexpression of genes responsible for root cell wall thickening and cell wall POD to prevent excess Fe uptake and stress.	Mehrabanjoubani et al. (2019)
/	*Oryza sativa* L.	Up-regulation of genes involved in the synthesis of photosynthetic transport chain proteins under high zinc stress	(Song et al., 2014)
/	*Cucumis sativus* L. and *Arabidopsis*	Increased expression of Cu/Zn SOD isoforms (CSD1 and CSD2) under Cu excess	(Bosnić et al., 2019; Khandekar and Leisner, 2011)
/	*Capsicum annuum*	Overexpression of silicon transporters (Lsi1 and Lsi2) mediating the transport of phytohormones (jasmonic acid, auxin, gibberellin, salicylic acid) promoting plant development under stress conditions.	Gómez-Merino et al. (2020)
/	*Oryza sativa* L.	Overexpression of DREB2A and NAC5 under water stress.	Khattab et al. (2014)

Under both water and salt stresses, Si helps to increase root water absorption, balance mineral content, and synthetise compatible osmolytes (Hidalgo-Santiago et al., 2021). Salt-sensitive okra (*Abelmoschus esculentus*) genotypes and *Capsicum annuum* cultivars accumulate proline when treated with Si under salinity (Abbas et al., 2015; Soares Pereira et al., 2013). Whereas in soybean and wheat, Si mitigate the salt stress effects so as to induce a decrease of proline accumulation (Bybordi, 2015; Lee et al., 2010). Probably the aforementioned upregulation of aquaporins activity may increase the rate of water uptake diluting the excess of Na^+ ions toxic for the plants (Rios et al., 2017). Moreover, Si has been found able to reduce Na^+ uptake in rice even if little is known about its effects on Na^+ flow and partitioning (Mir et al., 2022; Yeo et al., 2002). The higher capacity of Si treated plants to uptake and discriminate nutrients may depend on the well-known effect of Si to be incorporated in the plant cell walls, together with metal cations, forming a polymer called silica (silicon dioxide, SiO_2) thus rendering the cell walls more rigid. Si deposition contributes to remodel and increase the rigidity of the cell walls of stele and endodermal tissues thus strengthening the root mechanical barriers, whereas in the apical and subapical zones it enhances the extensibility of cell walls (Mehrabanjoubani et al., 2019). Indeed, it can affect the expression of genes involved in cell wall synthesis in order to modify its structure, strengthen and extensibility. In rice, Si supply enhances the lignification of the exodermis and endodermis at 4–5 cm from root tips by increasing the expression of genes coding for phenylalanine ammonia lyase (PAL) and 4–coumarate: CoA ligase (4CL) enzymes, essential for phenylpropanoid, and in particular monolignol, synthesis. The involvment of Si also in the induction of transcription of ABC transporters, involved in plant response to abiotic stresses by trasporting xenobiotics and endogenous secondary metabolites through extra- and intracellular membranes, and peroxidases (POD) makes possible the transfer of monolignols to apoplast and their polymerization in lignine by apoplastic PODs (Fleck et al., 2011; Mehrabanjoubani et al., 2019). The strenghtening of cell walls makes not only plants more resistant to mechanical stresses but also helps stabilizing cell membranes, thus enhancing nutrient uptake and transport (in particular that of nitrogen, phosphorus, potassium, and calcium) without necessarily upregulating mineral transmembrane transporters. This improvement of nutrient uptake capacity ameliorates plants performance in terms of growth, metabolism and stress resistance (Sheng et al., 2018; Wang et al., 2021). Stiffer and thicker cell walls in coffee seedlings due to Si treatment allowed also leaves to be more rigid and herect thus reducing the adaxial angle of the first leaves, and improving light penetration in the canopy (Yang et al., 2022). Si has been found also able to boost photosynthetic process in Zn stressed rice plants by upregulating the expression of the genes *PsbY*, coding for a subunit of PSII oxygen-evolving complex having a Mn-binding site, *PsaH* coding for a subunit of PSI, PetH coding for ferredoxin-NADP$^+$ and PetC related to cytochrome . Si treatment has also been found able to enhance the expression of PSII *PsbH, PsbB*, and *PsbD* genes in wheat; PsbH has a role in modulating PSII assembly and stability and in protecting the PSII core and the thylakoid membrane from oxidative damage; *PsbB* gene (Song et al., 2014) encodes the photosystem II (PSII) chlorophyll-binding protein and *PsbD* encodes the protein D2 of the reaction center of PSII (Hassan et al., 2021). The Si-dependent upregulation of the aforementioned genes has the capacity to enhance the Mn-binding capacity, the rate of oxidation of water and the electron transfer rate both at PS II and PSI levels thereby improving the efficiency of photosynthesis.

Si is able to improve nutrients uptake and photosynthesis efficency under drought or salt stress, and enhance the synthesis and accumulation of phenolic compounds thus boosting primary and secondary metabolism and antioxidant response.

Moreover, Si is involved in the restrictions of HMs uptake and translocation and their sequestration, mainly by altering soil pH, forming complexes with them in the soil or in the plants and co-precipitating or compartimentalizing them (Debona et al., 2017). In rice under HMs stress, Si reduced the uptake of toxic metals, exerting a downredulation of expression of genes encoding HM transporters *HMA2* and *HMA3*, while upregulating genes responsible for Si transport *LSi1* and *LSi2* (Bhat et al., 2019). Si ameliorated the root damages caused by HMs (e.g., Cd/Cu) also reducing lipid peroxidation and fatty acid desaturation in plant tissues, demonstrating to act on HMs uptake and/or compartimentalization and oxidative stress defence. Si, in fact, increased the contents of Cu/Zn SOD isoforms CSD1 and CSD2 and SOD activity in roots and shoots of cucumber seedlings when supplied to plants under Cu excess (Bosnić et al., 2019). Also in *A. thaliana*, Si upregulated the expression of *CSD1* and *CSD2* genes to cope with Cu toxicity (Khandekar and Leisner, 2011).

Si has been shown to induce the expression of stress-responsive genes, which are involved in mitigating the negative effects of abiotic stresses on plants. In particular, Khattab et al. (2014) found that the supply of Si in rice could up-regulate the expression of DREB2A and NAC5 (TFs specifically binding *cis* elements responsive to drought and low-temperature stress), *Oryza sativa* RING domain containing protein (OsRDCP1) (a RING E3 ligase responsible for the transportaion or degradation of TFs inhibiting the expression of water stress-induced genes), choline monooxygenase (CMO), and dehydrin OsRAB16b decreasing cellular osmotic potential. Hao et al. (2021) performed a RNA-seq analysis in wheat plants showing that 1164 genes were up-regulated and 1892 genes down-regulated by Si treatment, respect to control plants. The majority of differentially expressed genes coded for proteins involved in secondary metabolism and TFs, in particular R2R3-MYBs families that represent 14.66 % of the total differentially expressed TFs, which regulate plant growth, development and confer resistance to infection to pathogenic fungi in plants.

Si can influence metabolism and synthesis of phytohormones like ABA, jasmonic acid (JA), gibberellines, ethylene, salicilic acid (SA), brassinosteroids (BR), and auxins (Arif et al., 2021), to boost physiological responses of crop plants to extreme environmental conditions (Kim et al., 2016). In pepper, Si enhanced the synthesis of phytohormones modulating the growth, metabolism, and stress tolerance (Gómez-Merino et al., 2020). Particularly, in different experiments done on rice under HMs stress, Si upregulated the ABA biosynthtic pathway while reduced that of SA and JA (Mir et al., 2022).

Some studies suggested that Si could also impact gene expression through epigenetic mechanisms. By methylation-sensitive amplification polymorphisms (MSAP), Stadnik et al. (2023) analysed the effect of foliar application of Si on oat plants under 200 mM NaCl salinity. The results showed that exogenous application of silicon at 0.2 % contrasted the reduction of total DNA methylation exterted by salinity, limiting the alteration of the chromatin structure by chromatin remodeling and/or histone modification thus increasing plant tolerance to salinity.

6. Conclusions

The current need to steer agriculture toward greater independence from external chemical inputs has led the entire agricultural sector to seek sustainable, but equally efficient solutions. The introduction of biostimulant substances could represent a viable, albeit not necessarily definitive, solution for the future of agriculture. Consequently, the scientific community has placed significant emphasis on the use of products containing biostimulant substances, especially in light of the promising results obtained in sub-optimal conditions, which will increasingly characterize ordinary situations. Although biostimulants encompass both microbial and non-microbial origin products, we have chosen to focus our attention on protein hydrolysates, algae extracts, humic acids, and silicon. Although the literature has mainly addressed the management of biostimulants, such as the timing and method of application, to assess the true potential of these products, this approach remains too empirical and subjective. The overview presented in our review highlights the need for interdisciplinary research on biostimulants because their positive effects on the primary and/or secondary metabolism of plants are profoundly influenced by the synergistic action of numerous bioactive components. Therefore, a systematic molecular characterization of the different products becomes essential, as only in this way can their real potential be fully understood. The support of genomic, metabolic, and physiological analyses would further deepen our understanding of often overlooked action mechanisms under optimal conditions. In the future, basic research on biostimulant products should begin with the most detailed observations to justify and comprehend significant transformations. This strategy would enable researchers to develop tailor-made formulations to support global agriculture.

However, before using and/or commercializing a new biostimulant product, it is necessary to comply with the regulations and guidelines for the use of biostimulants to prevent unwanted consequences and ensure the safety of both consumers and the environment. In fact, to unravel the nature and risk profile of the new biostimulants, their different component materials are subjected to extra criteria and limits, following the roles for REACH (short for the Registration, Evaluation, Authorisation and Restriction of Chemicals, Regulation (EC) 1907/2006) as referenced in the Fertilizing Products Regulation (EU) 2019/1009 for relevant component materials, even if the new standard itself does not include a list of toxicological and ecotoxicological data. Moreover, the interaction of biostimulants with other agrochemicals, such as fertilizers or pesticides, are considered to avoid negative synergies or interference. As reported by Kumari et al. (2022) the application of legally approved biostimulants does not cause dangerous effects on the environment and mainly provides nutrients to plants. Indeed, farmers need to assess the economic feasibility of incorporating biostimulants into their agricultural practices, considering the potential costs and benefits.

CRediT authorship contribution statement

Michele Ciriello: Writing – review & editing, Writing – original draft. **Giovanna Marta Fusco:** Writing – original draft. **Pasqualina Woodrow:** Writing – original draft. **Petronia Carillo:** Writing – original draft, Validation, Supervision. **Youssef Rouphael:** Supervision, Project administration.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

No data was used for the research described in the article.

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Supplementary materials

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