*horticulturae*

*Review*

# The Role of Organic Extracts and Inorganic Compounds as Alleviators of Drought Stress in Plants

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**Abstract:** Climate changes have exacerbated the progression of drought conditions on a global scalethreating to crop production and heightening concerns over food security. Water scarcity enforces alterations in fundamental morphology, physiology and biochemical traits in crops. Consequently, it is imperative to identify environmentally sustainable alternative solutions to mitigate this problem and enhance overall plant performance. In this sense, biostimulants have emerged as a promising alternative as they improve plant resilience, enhance physiological processes, and mitigate the detrimental consequences of water deficit conditions on crop production. This review compiles the latest research on the application of organic extracts and inorganic compounds in crops subjected to drought conditions, specifically humic acids, protein hydrolysates, seaweed extracts, and silicon. Moreover, it offers a comprehensive overview of the origins and effectiveness of these biostimulants, with a detailed analysis of their application and the associated physiological, biochemical, and genetic modifications induced by these bioactive compounds. This knowledge enhances the understanding of the efficacy and implementation strategies pertinent of these compounds under water stress scenarios in agricultural settings.

**Keywords:** humic acids; protein hydrolysates; silicon; seaweed extracts; water scarcity



Academic Editor: Thomas E. Marler

Received: 26 November 2024
Revised: 10 January 2025
Accepted: 11 January 2025
Published: 15 January 2025

**Citation:** Garcia-Caparros, P.; Ciriello, M.; Rouphael, Y.; Giordano, M. The Role of Organic Extracts and Inorganic Compounds as Alleviators of Drought Stress in Plants. *Horticulturae* **2025**, *11*, 91. https://doi.org/10.3390/horticulturae11010091



## 1. Introduction

In recent years, changes in climate patterns have exerted a significant influence on agricultural regions, particularly manifesting notable impacts in arid, semi-arid, and coastal regions [1]. Currently, approximately one-third of arable lands are categorized as arid or semi-arid regions, with the severity of drought exhibiting an escalating trajectory. Drought is a major abiotic stressor, exerting profound detrimental effects on crops worldwide [2,3]. Forecasts predict a notable increase in mean air temperature by 5 °C in forthcoming years, further exacerbating the prevalence of drought occurrences and intensities [4,5].

The negative impact of drought stress on plants is contingent upon both the intensity and duration of the stress, with its severity intricately linked to the developmental stage of the plant. Drought stress elicits a spectrum of effects on plants at multiple levels of biological organization, encompassing anatomical and biochemical aspects [2,6] (Figure 1).

In terms of morphology, the impact of water scarcity on crops is manifested by observable reductions in plant growth and hastened leaf senescence. These conspicuous phenotypic changes culminate in a pronounced deterioration in both the quality and quantity of yield, serving to underscore the profound deleterious effects of drought stress on agricultural productivity [7,8].

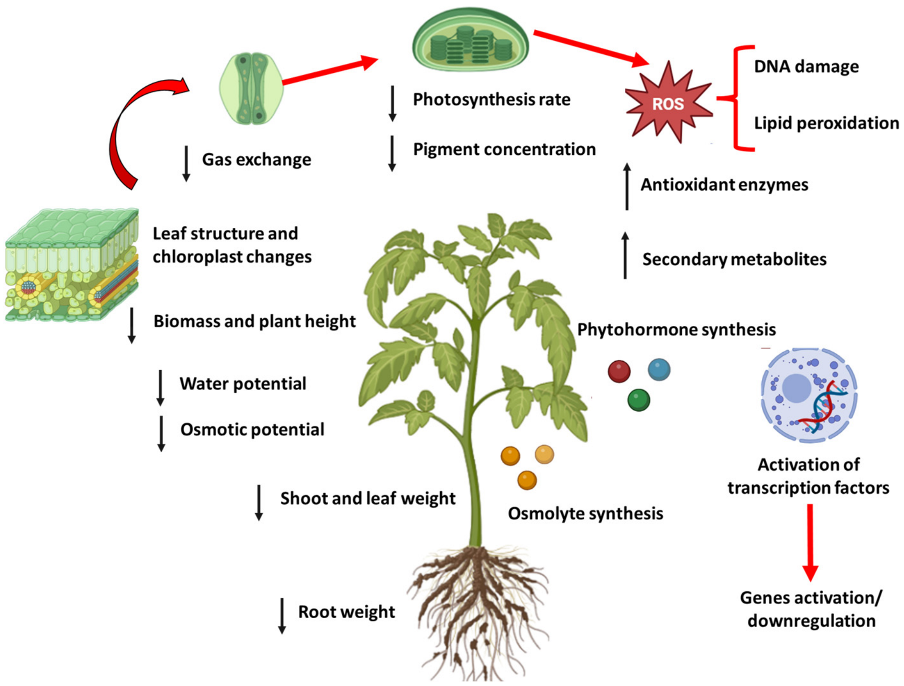


**Figure 1.** Water stress-induced plant anatomical and biochemical changes. The figure was generated using BioRender software (https://www.biorender.com/, accessed on 11 July 2024).

At the physiological level, water stress "pushes" plants to close their stomata, a pivotal mechanism aimed at reducing water loss by transpiration and thus conserving water resources. This response inevitably limits the diffusion of carbon dioxide ($CO_2$) into the leaf for photosynthetic assimilation. Furthermore, drought stress disrupts the hydraulic conductivity of plants, impeding the upward movement of water from roots to shoots. This disruption not only compromises the transport of water and essential nutrients but also disturbs the delicate balance of osmotic regulation within plant tissues [9,10].

At the biochemical level, the imposition of drought-induced water deficits triggers an elevation in the production of reactive oxygen species (ROS) within plant cells. This surge in ROS levels instigates oxidative stress, which can inflict damage on cellular structures and biomolecules. In response, plants activate an array of antioxidant defense mechanisms to scavenge excess ROS and mitigate oxidative injury, thereby preserving cellular integrity and function [11,12].

At the genetic level, drought stress can induce the overexpression or downregulation of several functional and regulatory genes. Functional genes are linked to the resistance against environmental stress, such as aquaporin genes, LEA proteins genes. In contrast, regulatory genes are focused on signal transduction and regulation of gene expression, enabling an indirect response to stress. These include genes encoding protein kinases, protein phosphatases genes, and other signaling molecules [6].

In the face of the escalating challenges posed by drought stress on crop productivity, the strategic application of biostimulants emerges as a compelling imperative. These substances and/or microorganisms offer a multifaceted approach to strengthen plant resilience, enhance physiological processes, and ameliorate the detrimental consequences of water deficit conditions on crop production. Under European Community Regulation (EU) 2019/1009 [13], plant biostimulants have been delineated based on four distinct claims: "Plant biostimulants are EU fertilizer products intended to stimulate plant nutrition processes regardless of the nutrient content of the product. Their primary objective is to improve one or more of the following plants and/or rhizosphere characteristics: (1) nutrient use efficiency, (2) tolerance and resistance to biotic and abiotic stressors, (3) quality attributes, or (4) availability of nutrients confined in the soil or rhizosphere. Under the current regulatory framework, biostimulants are subject to classification, a fundamental process designed to delineate their various constituents and functional attributes. Within this framework, biostimulants have been systematically categorized into two principal groups: microbial and non-microbial. The microbial classification encompasses organisms such as beneficial fungi and bacteria, while the non-microbial category encompasses a broad spectrum of substances, including plant and seaweed extracts, biopolymers, protein hydrolysates, amino acids, humic acids, and minerals. This classification schema is integral to regulatory coherence and scientific elucidation within the realms of agricultural and environmental jurisprudence, offering a structured framework for the assessment and management of biostimulant products [13].

The effectiveness of nonmicrobial biostimulants is mainly attributed to their rich repertoire of bioactive compounds, particularly amino acids and phytohormones. These constituents exert pronounced effects on plant growth dynamics by intricately modulating primary metabolic pathways. They also play a pivotal role in orchestrating secondary metabolic processes within plants [14,15].

Originally confined primarily to organic agricultural practices, plant biostimulants have undergone a notable expansion in their utilization, permeating various cropping systems, including conventional and integrated crop production methodologies. This evolution highlights a fundamental shift in the perception and application of biostimulant technologies, signifying their broader recognition and acceptance within the agricultural community. Such widespread adoption signifies a pivotal shift in agricultural practices, wherein biostimulants are increasingly acknowledged as valuable tools for enhancing crop productivity and sustainability across diverse farming paradigms [16].

According to analysis by Traon et al. (2014) [17], Italy, France, and Spain are the leading producers of biostimulants in the world. The market analyses revealed that during the period from 2016 to 2021, the global biostimulant market had a compound annual growth rate (CAGR) of more than 10%. One of the issues still poorly discussed concerns the analysis of the influence of biostimulant treatments on the production and economic structure. In any case, while the use of biostimulants, regardless of their mode of application, represents a cost, the possibility of increasing yield would improve the economic efficiency of the farm. These economic benefits would increase even more there where the application of biostimulants would lead to a significant reduction in utulized inputs such as nutrients and water.

Recent reviews on the role of non-microbial biostimulants in mitigating drought stress [18–27] have examined scientific reports and book chapters published in recent years. These reviews summarize the potential role of non-microbial biostimulants in mitigating the effects of climate change on crops.

Despite these insights, the mechanism of action of biostimulants remains unclear and is only hypothesized. We believe it is essential to study the multifaceted actions of biostimulants to understand their effectiveness against stress, much like assembling pieces of a puzzle. Our review is innovative because we are among the first to detail the physiological, molecular, and genetic mechanisms of non-microbial biostimulants in mitigating water stress. Additionally, unlike many reviews that focus on a single crop, we have addressed various crops, including herbaceous, fruit trees, vines, and vegetable species.

The objective of this review goes to elucidate opportunities that could be effectively exploited by applying nonmicrobial biostimulants to increase plant resilience to water stresses often associated with climate change.

## 2. Humic Acids

### 2.1. Origin and Effectiveness of Humic Acids as Biostimulants in Agriculture

Humic substances (HS) are the product of intricate chemical and biological processes involving the incorporation of organic materials derived from plant and animal residues, along with microbial metabolism. Humic substances represent the predominant reservoir of organic carbon within Earth's terrestrial ecosystems. Constituting over sixty percent of soil organic matter, they serve as a fundamental component in organic fertilizers. For this reason, HS are recognized for their significant nutrient content [28]. Humic substances, integral components of soil and natural organic matter, are traditionally categorized into distinct classes, namely humic acids (HA), fulvic acids (FA), and humins. This classification is mainly based on the solubility behavior of these substances in aqueous environments [29]. Due to their chemical reactivity, ability to resist microbial interactions, and lower degradation, researchers have turned their focus to humic acids for their remarkable ability to improve fertility and promote soil health in a relatively short time [30]. The structure of HAs comprises numerous functional groups, with phenolic (OH) and carboxylic (COOH) groups being predominant [29].

Humic acids represent a constituent of organic matter serving as a precursor to humic compounds, and they exhibit solubility under acidic conditions. The presence of several functional groups in humic acids results in unique characteristics that can promote plant development by inducing carbon uptake and metabolism [30]. In addition to its role in carbon cycling, the utilization of HAs has been shown to augment nitrogen metabolism by enhancing the activities of key enzymes such as nitrate reductase (NR), glutamate dehydrogenase (GDH), and glutamine synthetase (GS), all of which are integral to nitrogen assimilation pathways [31].

At the soil level, HA supplementation enhances the physicochemical properties of the soil, improving its structure, texture, microbial abundance, water-holding capacity (WHC) and soil nutrient availability [32]. As a result, root growth is stimulated, promoting the exudation of molecular weight organic anions by roots, which culminates in the release of soil micronutrients such as Fe, Mn and Zn [33]. Humic acids have been observed to facilitate crop growth through a myriad of metabolic mechanisms. These include increased cell membrane permeability, enhanced mineral assimilation, elevated rates of photosynthesis and respiration, and enhanced protein synthesis and hormonal activities [34]. The increased promotion of root and leaf growth and development has a considerable impact on the commercial quality and market value of plant products [35,36]. The impact of HA on soil and crop dynamics is contingent upon the specific source of HS utilized [30]. The selection of an HA source is predicated upon a multitude of factors, including its nutritional composition, method of production, functional group distribution, and intended application purpose. A comparative analysis of five distinct HA sources, scrutinized for their efficacy in influencing crop agronomic parameters, revealed a hierarchical trend in their effectiveness. Notably, the observed order of effectiveness delineated from highest to lowest efficacy includes compost derived from manure, compost sourced from green waste, native soil HS, HAs derived from brown coal, and those derived from peat, reviewed by Sible et al. [37].

### *2.2. Morphological, Physiological, and Biochemical Changes Induced from Humic Acids to Mitigate Drought Stress in Agriculture Crops*

Drought stress poses a significant threat to global agricultural productivity, necessitating the exploration of innovative strategies to mitigate its adverse effects. Among these strategies, the application of humic acids has garnered attention due to their multifaceted beneficial effects on plant growth and stress tolerance. The application of HAs has been correlated with discernible morphological alterations in plants experiencing drought stress. Their ability to augment stomatal conductance and improve water use efficiency contributes to the amelioration of water loss and the preservation of cellular hydration status [38]. Additionally, HAs have been demonstrated to upregulate the enzymatic scavenging of ROS, thereby enhancing the antioxidant defense mechanisms within plant cells [39,40].

A plethora of scholarly research papers have delved into the utilization of humic acids to ameliorate physiological and biochemical responses in diverse crop species under water scarcity conditions, as delineated in Table 1. Predominantly conducted in open field environments, these investigations have rigorously examined the application modalities of HA products, encompassing both irrigation and foliar spraying techniques. Such methodological diversity underscores the meticulous approach adopted in elucidating the potential of humic acids to bolster crop resilience, particularly against the backdrop of drought stress, within agricultural contexts.

Kiran et al. [41] investigated the impact of drought stress on *Cucumis melo* cultivated under greenhouse conditions. Plants were subjected to drought stress (100% and 50% of field capacity irrigation) from 35 to 77 days after seed sowing. Aiming to mitigate the effects of water scarcity, plants received liquid humic acid at a dose of 2000 mg $L^{-1}$ applied via irrigation. The findings obtained revealing an increase in leaf SOD, CAT, and GR activities and a reduction in leaf $H_2O_2$ concentration.

Forotaghe et al. [42] assessed the performance of onions cultivated under greenhouse conditions and subjected to three different levels of water stress (80, 70 and 60% field capacity). Drought stress was imposed during both vegetative and reproductive stages. To mitigate the adverse effects of water scarcity, solid humic acid powder (1 g per pot) was applied. The results demonstrated that onion plants showed increased leaf protein content as well as enhanced SOD and POD activities.

### *2.3. Genes Involved in Drought Tolerance in Agriculture Crops Treated with Humic Acids*

The application of HAs in agriculture has been proved to alter genes expression in crops contributing to various physiological and biochemical changes that enhance plant growth, stress resilience, and yield. Stress-responsive genes, nutrient uptake genes, hormone-related genes, defense-related genes and genes expression networks can be stimulated under the application of HAs [38]. Although changes in plant physiology and metabolism have been well documented in crops subjected to drought stress and humic acid application, the paucity of literature on transcriptomic studies has led to further research to discern the role of genes in orchestrating the plant response to stress. In this comprehensive review, we have collected, with respect to studies conducted on humic acid-treated plants, references from the existing literature including stress-responsive genes, transcription factors, and genes associated with photosynthesis and growth regulation, briefly presented in Table 2.

**Table 1.** Drought stress physiological and biochemical changes in agriculture crops treated with humic acids.

| Humic Acids Description | Crop | Growing Conditions | Drought Stress Treatment | Plant Growth Stage at the Stress Treatment Application | Effects of Humic Acids on Stressed Crops | References |
|---|---|---|---|---|---|---|
| Humic acid ($C_9H_9NO_6$, seeds soaking for 12 h (0, 50, 100, 200 and 300 mg $L^{-1}$)) | *Setaria italica* Beauv. | Potted experiment under field conditions | 3–5 leaf stage (set as 0 d), water application was stopped | 10 d after drought treatment | Reduction in $H_2O_2$ and MDA content and SOD and POD activities increase, | [34] |
| Humic acid (5 mM) applied via fertigation | *Fragaria ananassa* | Glasshouse | 100, 70 and 40% field capacity | From 4 weeks after sowing until 12 weeks after sowing | Increase in Chl content, reduction in leaf proline and MDA content | [43] |
| Humic acid (as 3-ethoxy-4-hydroxybenzaldehyde (foliar application (dose 5 mL per plant)) at 1 mM | *Zea mays* L. | Hydroponic | 10% (*w*/*v*) PEG-6000 to achieve −0.15 MPa osmotic potential | Only 18 days | Reduction in electrolyte leakage and increased leaf membrane stability. Increase in pigments concentration. | [44] |
| Humic acid foliar application at different concentrations: 0, 3, and 6 mg $L^{-1}$) | *Brassica napus* L. | Field | Drought treatments: 60, 100, and 140 mm evaporation from class A pan | Vegetative and early flowering stages | Increase in APX and POD activities and MDA level and soluble protein content | [45] |
| Humic acid application at 0, 2, 4 and 6 L per ha | *Zea mays* L. | Field | Three irrigation levels after depleting 30, 40 and 50% of field capacity (optimum irrigation, moderate stress and severe stress) | From 30 days after planting to 25 days before harvest (60 days approximately) | Higher yield and SOD and CAT activities | [46] |
| Humic acid foliar application (0, 150 and 300 ppm) | *Triticum aestivum* | Field | Complete irrigation, irrigation withholding at stem elongation stage, irrigation withholding at flowering stage and irrigation withholding at seed setting stage | Stem elongation, flowering and seed setting stages | Higher SOD and GPX activities and lower MDA content | [47] |

**Table 1.** *Cont.*

| Humic Acids Description | Crop | Growing Conditions | Drought Stress Treatment | Plant Growth Stage at the Stress Treatment Application | Effects of Humic Acids on Stressed Crops | References |
|---|---|---|---|---|---|---|
| Humic acid, foliar application in the volume of 4.5 L per one thousand liters of water | *Oryza sativa* | Field | Well-watered conditions, water restriction at the tillering stage and grain filling stage | Tillering and grain filling stages | Lower leaf proline content, reduced CAT activity and increased APX activity | [48] |
| Humic acid application through irrigation water (0 and 4 kg $ha^{-1}$). two times during vegetative growth of roselle (15 and 35 days after emergence). | *Hibiscus sabdariffa* L. | Field | Water regimes [irrigation after pan evaporation of 100 mm (normal irrigation) and 200 mm (deficit irrigation)] | After the first week of seed sowing until the harvest (approximately 7 months) | Enhanced acidity and maturity index in calyx of roselle | [49] |
| Humic acid application through irrigation water (0 and 4 kg $ha^{-1}$). | *Zea mays* | Greenhouse | 100 and 60% water holding capacity | 28 days after seedlings establishment | Enhanced photosynthesis by increasing the electron transport rate (ETR) of photosystem II (PSII) and the ratio between effective photochemical quantum yield to non-photochemical quenching (Y(II)/Y(NPQ) | [50] |
| Humic acid drenched in the soil (0, 250, 500 and 1000 mg $kg^{-1}$) | *Echinacea purpurea* | Field | 100, 80, 60 and 40% field capacity | After three months of sowing | Enhanced relative water content, electrolyte leakage reduction and higher content of total phenolic and flavonoid content in shoot | [51] |

**Table 2.** Drought stress responsive genes in agriculture crops treated with humic acids.

| Biostimulant | Crop | Growing Conditions | Drought Stress Treatment | Genes Activated by Drought Stress | References |
|---|---|---|---|---|---|
| Liquid phase, rate of 60 Kg ha$^{-1}$ in 3 equally doses (first in germination, second two weeks later and third: initiation of flowering) | *Vigna radiata* | Field | No irrigation after 15 days from the vegetative stage until the reproductive stage | Upregulation of VrHsfA6a genes and VrDREB2a, and VrBZIP17 transcription factors | [52] |
| Foliar spray of HA at increasing concentrations (50, 100, 200, 300, and 400 mg L$^{-1}$) | *Setaria italica Beauv.* | Field | Dry region simulating a drought environment | Upregulation of SETIT_021707mg, SETIT_016840mg, and SETIT_015030mg genes and downregulation of SETIT_004913mg and SETIT_016654mg genes | [34] |
| Drenching into the soil with two different rates: 0 and 45 Kg ha$^{-1}$ | *Zea mays* | Field | Drought stress (W1, 45–60% soil water holding capacity (SWHC)) and well-watered (W2, 75–100% SWHC). | Upregulation of psbQ and psbP genes (encoding the extrinsic proteins of PS II complexes) and genes involved in the Calvin cycle regulation | [53] |
| Foliar spray of HA at 1% *w*/*v* | *Triticum aestivum* | Hydroponic | After the 7th day of drought stress treatments (MD, moderate drought (−6 bar PEG6000); HD, high drought (−8 bar PEG6000) | Downregulation of miR396-targeted growth-regulating factor (GRF) and AP2 gene (miRNA Apetala 2) in root and upregulation in leaf | [54] |

## 3. Protein Hydrolysates from Vegetal and Animal Sources

### 3.1. Origin and Effectiveness of Protein Hydrolisates as Biostimulants in Agriculture

Protein hydrolysates are mixtures consisting mainly of free amino acids and peptides and, in a small percentage, carbohydrates and minerals. They are obtained by chemical and/or enzymatic hydrolysis of animal and plant proteins often from agrifood by-products (e.g., blood, viscera, plant residues, etc.). The possibility of valorizing waste from other sectors makes these biostimulants attractive from an economic and environmental circularity perspective [55,56]. The most used modes of application are foliar and root applications. As reported by Paul et al. [57], foliar applications respond in a short time, while root applications have a long-term effect. In any case, it has been found that the application method differentially regulates ammonium and nitrate transporter genes and some nitrogen metabolism genes in tomato plants [58]. They can act directly on the plant, or their action is modulated through interaction with soil microorganisms [59]. Furthermore, protein hydrolysates can have direct action on carbon and nitrogen metabolism, as they activate enzymes involved in the absorption and assimilation of nitrate, the Krebs cycle and glycolysis, and an indirect action on nutrient-use efficiency, through a change in the root system (increase in root length, thickness, number of lateral and secondary roots) [60]. Like other plant biostimulants, their action appeared to be most efficient under suboptimal growth conditions [58,61].

As observed by Trevisan et al. [62] exogenous application of protein hydrolysate regulated nitrogen uptake and antioxidant defense of *Zea mays* L. plants grown under salt stress and hypoxic conditions. Similarly in lettuce plants subjected to high salt stress, improved photosynthetic activity and increased osmolytes and nitrogen metabolism were recorded following the application of a plant-derived protein hydrolysate [63]. These studies are confirmed by the positive effect of specific exogenous amino acids and peptides on the physiological processes of several plants under abiotic stress [64–66]. Several grapevine crops have improved their resistance to fungal attacks (for example, *Botrytis cinerea*, *Plasmopara viticola*) due to an increase in secondary metabolites such as anthocyanins, resveratrol, polyphenols, in leaves and fruits, induced by treatment with protein hydrolysates [67–71]. The aforementioned results have demonstrated that protein hydrolysates can act at both the primary and secondary metabolism levels, activating physiological and molecular mechanisms that allow plants to defend themselves against different types of stress. The ability of protein hydrolysates to mitigate the growth reduction induced by different types of stress is due to their ability to modify the metabolism of phytohormones, to activate signals, such as the increase in calcium ions in the cell, or proteins and molecules involved in the stress response, which increase the plant's tolerance to stress [72], Wang et al., 2022 [73]; Paul et al., 2019 [57]; Bavaresco et al., 2020 [74].

Some studies have shown that plants exposed to low concentrations of natural or synthetic substances can respond efficiently to a subsequent stress event. This practice is called priming and allows the plant to activate a defense before the stress has appeared, so that it quickly implements its response to stress [75].

Given the potential of biostimulants and the worsening of climate change today, this review aimed to highlight the effect of protein hydrolysates on drought stress.

### 3.2. Morphological, Physiological, and Biochemical Changes Induced from Protein Hydrolysates to Mitigate Drought Stress in Agriculture Crops

Drought stress affects all crops, especially those that require large quantities of water during their growth. Viticulture is among the first to be affected by drought, as drought can significantly reduce growth, physiology, production, and quality [76].

There are several attempts to increase grapevine tolerance to drought, and these concern optimal irrigation cycles [77], selection of more suitable rootstocks, genetic and biotechnological improvement.

Boselli et al. [71] examined the performance of water-stressed grapevines treated with three protein hydrolysates, obtained by enzymatic hydrolysis of soybean, lupin and dairy casein (Soy, Lup, Cas). The protein hydrolysates were applied three times during the growing seasons of Vitis vinifera L. cv. Corvina, over an experimental period of five years. The authors showed that application of the biostimulants significantly reduced stomatal conductance, a physiological response that would allow the plants to reduce transpiration demand and consequently better tolerate the imposed irrigation deficit [71,78]. However, the reduction in transpiration resulted in a significant increase in leaf temperature that may have triggered as suggested by Kauffman et al. [78] an early stress response through metabolic pathways related to abscisic acid (ABA) production. Not surprisingly, the reduction in stomatal conductance could be related to an anatomical change in leaves (such as number and size of stomata and cuticular thickness) induced by biostimulant application (Carillo et al., 2022; Vitale et al., 2021; Kirubakaran et al., 2007 [79–81]. The improved water management at the cellular level mediated by biostimulants applied under water stress conditions would justify the improved production performance recorded in treated plants.

Lachhab et al. [68] revealed the role of protein hydrolysates obtained from soybean and milk as activators of early response to water stress through increased abscisic acid, cytosolic calcium, and defense responses in grapevine cells.

Bavaresco et al. [74] investigated the effect of foliar application of two protein hydrolysates (Trainer® and Stimtide®) on the metabolism and protein profile of grapevine (*Vitis vinifera* L., cv Montepulciano) subjected to water stress and re-irrigation. Both biostimulants changed the metabolomic and protein profile of plants during stress, compared to the untreated control. The Trainer®, changed the concentration of 69 of the metabolites analyzed. Specifically, 19 were upregulated and 50 were downregulated. Those upregulated included adenine, which is a nucleic acid involved in various cellular processes, such as cell division, nitrogen absorption, cytokinin metabolism [82]. Furthermore, the two biostimulants increased the synthesis of (5-alpha)-campestan-3-one, a metabolite involved in the synthesis of brassinosteroids, which are phytohormones that regulate cell division and plant growth through modulation of auxins [83]. The increase of the brassinosteroids corresponded to the lowest leaf water potential (−1.4). Other upregulated metabolites belonged to the synthesis of waxes. Compounds involved in the biosynthesis of flavonols and flavonoids or their precursors were downregulated. 3-hidroxy-β-ionone, found increased with both biostimulants, is a compound involved in the cleavage of lutein and zeaxanthin, two carotenoids that epoxidize and deoxidize in a cycle that dissipates energy in the form of heat [84]. Among the physiological parameters examined (P, gs, E), transpiration measured five days after re-watering, was found higher in the leaves treated with the two biostimulants, compared to the control. Furthermore, at harvest (when the vines had a TSS concentration of 24 °Brix), the treatment with the two biostimulants produced a reduction in TSS in grapes, an increase in titratable acidity, and a reduction in pH, compared to the control. While anthocyanins and total phenolics did not differ between treatments.

Through a study on *Capsicum annum* L., Agliassa et al. [83] sought to understand whether the application of a protein hydrolysate could exert a priming action. To answer this question, the authors applied a plant-derived protein hydrolysate before a major stress event to highlight the action of the biostimulant in increasing stress tolerance and its priming action. The stress consisted of stopping irrigation until the stem water potential was less than −2 MPa. The stress was followed by the recovery phase, which consisted of irrigating the plants until they were brought back to a physiological condition similar to those that the plants had before the start of the stress, monitoring the gaseous exchanges and the water potential of the stems every day. In conditions of severe stress, the plants treated with biostimulant (stressed-BIO) mitigated the stress, increasing plant growth (leaf area, height and diameter of the stems), compared to control plants (stressed-NO BIO). In the recovery phase, the plants treated with the biostimulant restored photosynthetic activity faster (1 day) compared to the plants not treated with the biostimulant (3 days). During recovery, the treated plants also had a higher stomatal density, a lower concentration of $H_2O_2$, and a higher activity of the catalase enzyme, compared to the stressed-NO BIO plants, Finally, the stressed-BIO plants presented a higher content of soluble sugars at the end of the stress and in the recovery phase. High levels of proline were detected at the end of the stress and in the first 4 h of recovery of the treated plants, which is probably an effective state for rapid recovery of stressed-BIO plants, A similar priming effect was shown for plants of *Vitis vinifera* L., Sauvignon blanc cultivar, grown in pots, were treated with a collagen-derived protein hydrolysate 48 days before progressive water stress (from 100% field capacity up to 30% field capacity, for 18 days) [85]. The protein hydrolysate mitigated water stress by supporting growth (internode length, leaf area), the water state of the cells (leaf water potential), and the increase of epigeal part of plants and berry diameter. Lysine, the most abundant amino acid in the protein hydrolysate used, is a precursor of glutamate, which is involved in growth and used as a signal molecule during stress [86,87]. Plants pre-treated with biostimulant before stress had a higher SPAD index compared to untreated plants. The SPAD index is a parameter related to the chlorophyll and nitrogen content of the leaves [88–91].

In the work of Francesca et al. [91], tomato plants (genotype 'E42') grown in open fields were treated with a protein hydrolysate of plant origin (CycoFlow), consisting of a mixture of sugar cane and *Saccharomyces cerevisiae* extracts, and applied every two weeks by fertigation (3 g $L^{-1}$), for a total of four applications. The biostimulant was rich in glutamic acid, glycine betaine, and micronutrients, such as boron, manganese, and zinc. The plants were grown in two different water regimes, one optimal (100% water) and one suboptimal (50% water). The water deficit state was applied 22 days after transplanting the plants, until the end of the crop cycle. The effect of water stress in plants not treated by the biostimulant was manifested by a notable reduction in pollen viability, number of fruits per plant, the average weight of the fruits, and yield., Under conditions of water stress, the biostimulant increased the values of pollen viability, number of fruits per plant, and the average weight of fruits, by 51%, 70%, and 95%, respectively, compared to untreated plants. Therefore, under conditions of water stress, the yield of the plants treated with the biostimulant reached 6 times the value of the untreated plants. The increase in pollen viability has been linked to the high concentration of β-alanine in the protein hydrolysate, which is considered a promoter of pollen germination in tomato plants subjected to high temperatures. In conditions of water deficit, stomatal conductance of biostimulant treated plants and control plants remained similar, while the water potential of biostimulant treated plants increased by 27% compared to the control plants leaves. In optimal water conditions, the biostimulant treatment reduced the content of ascorbic acid by 29%, the content of chlorophyll a and b by 14%, increased the content of carotenoids (+33%) and lycopene (+31%) compared to the untreated control. In conditions of water stress, biostimulant treatment increased leaves antioxidant activity (+98%), while reducing both total carotenoids (−20%) and lycopene content (−15%), compared to stressed and non-treated plants. Furthermore, ascorbic acid content of the treated plants did not differ from that of the untreated plants. The greater antioxidant power was probably due to the presence of a high concentration of molecules with antioxidant power already present in the biostimulant, such as glutamic acid, phenylalanine, glycine, and proline [92,93]. These molecules also play an important role as signaling molecules in endogenous hormonal pathways, thus supporting growth and productivity under stress conditions [51].

Wang et al. [64] examined the response of tomato plants treated with protein hydrolysate obtained from the enzymatic hydrolysis of pig blood, rich in peptides and free amino acids. Plants were grown in a controlled growth chamber, in plastic containers, and irrigated with Hoagland nutrient solution. At the phenological stage of 6 true leaves, plants were subjected to drought stress, which consisted of adding 10% PEG-6000 in the nutrient solution, compared with a control irrigated with the same nutrient solution, without stress. Both treatments were then divided into two parts: some plants were sprayed on the leaves with the protein hydrolysate at different doses (1, 2 and 3 g $L^{-1}$), while others received only water. Each treatment was applied three times before harvest. The authors showed a positive effect of protein hydrolysate on growth, chloroplast structure, chlorophyll content (a, b, total), photosynthetic activity and water-use efficiency in stressed plants. Furthermore, the protein hydrolysate increased the antioxidant defense in terms of enzymes (SOD, POD, CAT, and APX), and molecules (total phenolic, total flavonoid, ascorbic acid, and glutathione), with a consequent reduction of oxygen radicals, both in the leaves and in the roots.

An improvement in the mineral profile (K, Mg, Ca) in both leaves and stems and roots, and an increase in osmolytes (proline, sugars, and soluble proteins) were also observed. Sitohy et al. [94] attributed the tolerance of *Phaseolus vulgaris* subjected to salt stress to the increase in the mineral profile and osmolytes following the application of pumpkin seed protein hydrolysate. Protein hydrolysates have been shown to increase nutrient uptake by acting on specific root transporters [55,60,61], indirectly stimulating plant growth. A better performance of photosynthetic parameters in plants subjected to different abiotic stresses (hypoxia, salt, and nutrient deficiency) and treated with protein hydrolysate, was seen in *Zea mays* (L.) plants grown in hydroponics [62]. This result was attributed to the presence of amino acids in the biostimulant involved in the biosynthesis of chlorophylls, such as alanine, glycine, and lysine.

The activity of protein hydrolysates under drought conditions was examined by high-throughput phenotyping and metabolomic analysis of physiological and growth parameters of drought-stressed tomato plants grown in a controlled growth chamber by Paul et al. [48]. The biostimulant (protein hydrolysate obtained from legume seeds by enzymatic hydrolysis) was applied foliar (5 and 12 days after transplanting, 2 mL/500 mL of distilled water) or by soil soaking (4 mL $L^{-1}$). Both treatments increased tomato plant biomass and photosynthetic activity. There was a reduction in cytokinins and an accumulation of salicylic acid with both biostimulant treatments. Cytokinins are phytohormones that negatively regulate tolerance to water stress [95], so their reduction in treated plants is considered a positive aspect of the biostimulant action in stress conditions. Salicylic acid regulates the formation and accumulation of ROS in the plant [96], and together with jasmonate it increases tolerance to water stress [97]. The biostimulant treated plants in the work of Paul et al. [57] showed a better response in regulating the concentration of ROS, also due to the carotenoids and prenyl quinones increase, and reduction in tetrapyrrole coproporphyrins. Prenyl quinone is a chloroplast compound, with a signal molecule function and antioxidant activity, found to be involved in adaptation to stress [98]. Tetrapyrrole coproporphyrins is a molecule of the chlorophyll biosynthetic pathway that accumulates following cellular necrosis induced by excess light, it is involved in the formation of singlet oxygen following excess light [57].

Some of these results are summarized in Table 3.

**Table 3.** Drought stress physiological and biochemical changes in agriculture crops treated with protein hydrolysates.

| Protein Hydrolysates Description | Crop | Growing Conditions | Drought Stress Treatment | Plant Growth Stage at the Stress Treatment Application | Effects of Protein Hydrolysates on Stressed Crops | References |
|---|---|---|---|---|---|---|
| PHs from soybean (Soy), lupin (Lup) and dairy mix-based casein (Cas). PHs were sprayed three times every 10 days, at 0.5 and 2.0 kg $ha^{-1}$ | *Vitis vinifera* L. cv. Corvina | Field | Mediterranean climate (hot and dry summer, moderately cold and wet winter), compared to water-treated plants | Fruit set (BBCH 71) | Increase in yield, total soluble solids content, total anthocyanin content Reduction in stress index IG, water loss | [71] |
| Commercial Trainer and Stimtide obtained from an enzymatic hydrolysis legume biomass. PHs were sprayed 1 time per year at day of the year 201, at 3 mL $L^{-1}$ | *Vitis vinifera* L. cv. Montepulciano | Outdoor area. Plants grown into pots filled with a loamy soil and peat mixture (80:20) | Progressive water stress by shutting down irrigation, followed by plants re-watering | Four-year-old grapevines | Up regulation of metabolites involved in plant growth and photosynthesis-related proteins Improvement of leaf transpiration Increase of titratable acidity Reduction in total soluble solids and pH | [83] |
| Commercial GHI_16_VHL biostimulant obtained from *Cruciferae* and *Leguminosae* protein hydrolysates. PH was applied two times before the beginning of water stress, by fertigation (1.5 mL $L^{-1}$) | *Capsicum annuum* L. plants ("Corno di Toro Giallo" variety) | Greenhouse under partially controlled conditions. Plants growth into pots filled with sand:clay:peat (1:2:2 by weight) | Stopped irrigation until the stem water potential was below −2 Mpa. Followed by re-watering of plants. | 3-month-old plants | Faster leaf gas exchange recovery Increase in leaf area, height and diameter of the stems, CAT activity Reduction in hydrogen peroxide level | [85] |
| Novel collagen-derived protein thermal hydrolysate, APR® (0.5 g $L^{-1}$) added as a soil drench, and applied at flowers separating stage (day of the year 135). | *Vitis vinifera* L., cultivar Sauvignon Blanc | Plants grown in the tunnel with semi-controlled conditions, into pots filled with a sand–pumice-peat mixture (2:2:6 in volume). | Progressive water deficit starting at DOY 183, 48 days after biostimulant application, and for 18 days | 4-year-old plants | Increase in internode length, leaf area, berry diameter, leaf water potential | [86] |

**Table 3.** *Cont.*

| Protein Hydrolysates Description | Crop | Growing Conditions | Drought Stress Treatment | Plant Growth Stage at the Stress Treatment Application | Effects of Protein Hydrolysates on Stressed Crops | References |
|---|---|---|---|---|---|---|
| Novel protein hydrolysate-based biostimulant (CycoFlow) applied by fertigation (3 g $L^{-1}$ of water, 400 mL per plant) every 15 days until the end of the cultivation cycle | *Solanum lycopersicum* genotype 'E42' | Open field on a clay-loam soil irrigated every 10 days (5 L $h^{-1}$) | Limited water availability (50% irrigation) until the end of the experiment | 22 days after transplant | Increase in yield, water potential, pollen viability, number of fruits per plant, average weight of fruits, leaves antioxidant activity Reduction in total carotenoids and lycopene content | [92] |
| Pig blood-derived protein hydrolysate (PP, Win Plus) sprayed on the leaves at different doses (1, 2, and 3 g $L^{-1}$), three times before harvest. | *Solanum lycopersicum* | Plants growth in controlled chamber, into a plastic container with 1 L of Hoagland nutrient solution. | 10% PEG-6000 in the Hoagland nutrient solution | Tomato seedlings with six leaves | Increase in SOD, POD, CAT, APX activities, total phenolic, total flavonoid, ascorbic acid, and glutathione content, mineral profile (K, Mg, Ca) and osmolytes (proline, sugars, and soluble proteins) content | [73] |
| Commercial plant-derived PH biostimulant Trainer obtained from legume seeds, foliar supply (5 and 12 days after transplanting, 2 mL/500 mL distilled water) or soil drench (4 mL $L^{-1}$). | *Solanum lycopersicum* L.–cv. Hybrid F1 Chicco Rosso | Plants growth in controlled chamber, into pots filled with a mixture of a commercial peat-based substrate and river sand (3:1), and fertigated with a nutrient solution. | Moisture content adjusted to 60% of container capacity | Twenty-day-old plants | Increase in plants biomass, stomatal conductance Different regulation of phytohormones activity and lipids biosynthesis Reduction of cytokinins, and an accumulation of salicylic acid | [57] |

### 3.3. Genes Involved in Drought Tolerance in Agriculture Crops Treated with Protein Hydrolisates

Few studies in literature have been interested in understanding the gene mechanisms activated following the application of protein hydrolysates under water stress conditions. However, some works have reported the expression of genes encoding for secondary metabolites involved in defense against many abiotic stresses, such as drought, in plants treated with protein hydrolysates. An example is reported by Ertani et al. [99], who noted up-regulation of genes involved in detoxification processes from reactive oxygen species in tomato plants treated with alfalfa-based protein hydrolysate. Among these genes were glutathione peroxidase, glutathione reductase, peroxidases and thioredoxins. Most of these genes are involved in the glutathione/ascorbate detoxification cycle. In addition, hydrolysate-treated plants exhibited upregulation of genes involved in the biosynthesis of hormones such as ethylene, jasmonic acid, abscisic acid and salicylic acid. These hormones are involved in the phosphorylation of protein kinases which leads to the transcription of the abiotic stress defense-related genes. The authors also emphasize that the ethylene hormone, whose synthesis is stimulated by protein hydrolyzed, increased the expression of the gene that encodes the PAL enzyme, in the plants treated with the biostimulant. PAL enzymes are essential for the biosynthesis of many phenolic compounds used by plants against abiotic stress [100].

Xu et al. [101] showed that protein hydrolysates vary the expression of transcription factors involved in a multiplicity of transcriptional programs related to abiotic stress. AP2/ERBPS (APETALA2), WRKY, ZINC Finger (ZFN) proteins, and BZIP Proteins are some of these factors.

## 4. Seaweed Extracts

### 4.1. Origin and Effectiveness of Seaweed Extracts as Biostimulants in Agriculture

Macroalgae belong to Phaeophyta, Rhodophyta, and Chlorophyta classes, also known as brown, red, and green algae, respectively based on their color. Their use by humans has deep roots [102]. They have been used in medicine, cosmetics, and in agriculture as food to feed animals and as fertilizers, since the ancient Romans [102,103]. The use of algae extracts instead has more recent uses. They have been called plant biostimulants for their ability to promote plant growth and improve the nutritional aspect and shelf life [102]. The biostimulant action of algae extracts has not been attributed to their nutritional content (macronutrients) but to elicitor compounds capable of activating the physiological responses of the treated plants. Algae extracts regulate plant growth similarly to phytohormones as they stimulate, or slow down growth based on their concentration [104]. The phytohormone-like activity is due to the content of indole acetic acid, cytokinin, gibberellic acid, polyamines, and abscisic acid in the seaweed extracts [104,105]. They are rich in phenolic compounds with antioxidant activity [106], osmolytes such as mannitol and betaines, amino acids, vitamins [105]. They also contain polysaccharides [107] (alginates and laminarins) that promote plant growth and act as elicitors of plant defense against pathogenic infections [101,104]

The concentration of these substances and hormonal activity depends on the type of seaweed, seasonality, extraction method, and the type of processing they undergo [106].

Seaweed extracts are generally in liquid or soluble powder form. In liquid form, the extracts can be mixed into irrigation water and applied as drip irrigation to the crops, or as foliar sprays [102]. Seaweed extracts effectively depends on the growth stage of the plants and is highest when the stomata are open [102].

*Ascophyllum nodosum*, *Ecklonia maxima*, *Macrocystis pyrifera*, and *Durvillea potatorum* are the main brown macroalgae (Phaeophyta) used to produce extracts intended for agriculture and horticulture [104]. The main bioactive compound found in these macroalgae are summarized in the Table 4.

**Table 4.** Main brown macroalgae used as biostimulant.

| Species | Main Bioactive Compound | Cellular Action | References |
|---|---|---|---|
| *Ascophyllum nodosum* | Indoleacetic acid; Abscisic acid | Increase of roots number and length; regulation of stomata closure | Sanderson et al., 1987 [108] |
| *Ecklonia maxima* | 1-aminocyclo-propane-1-carboxylic acid; Abscisic acid | Precursor of ethylene synthesis, it promotes flowering and fruit ripening; regulation of stomata closure | Nelson and Van Staden, 1985 [109] |
| *Macrocystis pyrifera* | Molecules with auxin-like activity | Increase of roots, fruit setting | Briceño Dominguez et al., 2014; Colla and Rouphael, 2019 [110,111] |
| *Durvillea potatorum* | Fibres, alginic acid, laminarin, fucoidan, mannitol | Regulation of response to pathogens | Madgwick and Ralp, 1972; Di Stasio et al., 2018; Colla and Rouphael, 2019 [111–113]. |

Brown algae extracts are found to improve the soil water retention capacity, root growth and soil microbial activity [114,115]. Some extracts have modified the acidification activity of the plasma membrane proton pumps by inducing the secretion of $H^+$ ions, the rhizosphere, and increasing the solubility of some useful ions for plants [105]. Brown algae extract increased the absorption of copper, iron, calcium, potassium, and magnesium in grapevine, lettuce, cucumbers, and tomatoes, especially when the plants are in sub-optimal growth conditions or under environmental stresses [102]. Higher nitrogen and sulfur uptake were detected, too [116].

The bioactive compounds in the algae extracts are considered responsible for the increased tolerance to biotic and abiotic stresses of numerous crops [117]. *Ascophyllum nodosum* extracts applied to strawberry [118] and lettuce [119] plants allowed increased plant and root growth under salinity conditions. Yield and antioxidant defense increases were found in tomato plants grown in saline conditions and treated with *Dunaliella salina* extracts [120]. Chickpea plants treated with *Sargassum muticum* extracts had a greater tolerance to salinity due to the restoration of the ionic balance, a better antioxidant defense, and better regulation of the amino acids synthesis, compared to plants not treated with a biostimulant [121].

The *Padina gymnospora* seaweed extract improved the salinity tolerance of tomato plants due to the increase in photosynthetic activity, stomatal conductance, and the content of antioxidant enzymes [122]. *Brassica juncea* plants under thermal stress conditions had better growth and yield and less membrane impairment when treated with seaweed extract (3 mL $L^{-1}$ and 5 mL $L^{-1}$) [123]. The positive effect of seaweed extracts under salt stress conditions was also observed in pepper plants [124]. Extracts of *Ascophyllum nodosum* and *Sargassum* spp. sprayed on barley plants increased the plants' tolerance to cold through proline and non-structural carbohydrates increase, and osmotic adjustment [125].

A wide range of crops has been shown to increase chlorophyll content following treatment with algae extracts [116,126]. According to the authors, the increase in chlorophyll was linked to a high content in chloroplasts or a reduction in chlorophyll degradation [116,127]. According to some authors, the cytokinin-like activity of seaweed extracts induced the synthesis of cytokinins that imparted protection to chloroplasts [102,105]. The growth and concentration of photosynthetic pigments were found to be increased in cabbage [126] and *Spinacia oleracea* L. plants [127] treated with *Ecklonia maxima* extracts.

Seaweed extracts have antifungal properties against *Macrophomina phaseolina* (Tassi) Goid., and *Fusarium oxysporum*, blocking the growth of their mycelium [128]. The mycelial growth of four plant pathogenic fungi (*Botrytis cinerea*, *Aspergillus niger*, *Penicillium expansum*, and *Pyricularia oryzae*) was blocked using *Gracilariopsis persica* extract at 1000 µL [129].

Norrie et al. [130] examined the response of Thompson seedless grapes (*Vitis vinifera* L.) to the extracts of *Ascophyllum nodosum* in an experiment conducted over three years. The extract was applied as a spray at different stages: before and after flowering, before and during the sizing stage, during veraison, and in pre-harvest. For all three years of the experiment, the authors obtained a positive effect of the treatment on the total number of fruits, on the uniformity and weight of the berries, on the number of primary bunches, on the number of berries per bunch, with increases in yields, compared to untreated control plants.

However, the activity and mechanisms of action of algae and algae extracts on plants depend on various factors, such as the type of algae, the extraction mechanism, and the plant species [102]. For future studies, it would be interesting to understand the possible synergistic effect of extracts from different algae. Likewise, the plants stage should be understood to have the best benefits following the application of the extract.

### *4.2. Morphological, Physiological, and Biochemical Changes Induced from Seaweed Extracts to Mitigate Drought Stress in Agriculture Crops*

Broccoli [131] and spinach [132] plants treated with *A. nodosum* extracts had better resistance to drought stress due to an increase in gaseous exchange parameters, compared to untreated plants. Another symptom of drought stress is leaf yellowing caused by chlorophyll degradation. Extracts of *A. nodosum* have been shown to increase the chlorophyll content in tomato plants subjected to water stress [133]. Drought-stressed tomato plants had improved plant height, root length, and the number and area of the leaves [134] when treated with a microalgae-based biostimulant.

Extracts of *A. nodosum* reduced wilting, increased WUE, and accelerated recovery of several drought-stressed vegetables [102,135]. Extracts of *A. nodosum* also increased the water potential of almond plants under high-temperature conditions [130]. According to some authors, the cytokine-like activity and the increase in $K^+$ absorption induced in the plants treated by seaweed extracts explained the tolerance of creeping bentgrass to heat [102].

Foliar application of brown algae extract (*A. nodosum*) alleviated drought stress by increasing the synthesis of antioxidant enzymes, the accumulation of defense metabolites, and growth and sugar production in sugarcane plants [136].

Lenart et al. [137] applied marine algae extracts to 12 blueberry species grown in greenhouse pots under controlled stress conditions (the substrate was maintained at 40% field water). The authors showed an increase in the activity of antioxidant enzymes (peroxidase and catalase) in plants subjected to water deficit, compared to untreated control plants, with no differences in nutrient and chlorophyll content between treated and control plants. Similarly, Lenart et al., 2022 [138] showed that fertilization of blueberry fruit plants with algae increased the content of antioxidant molecules (anthocyanins and total polyphenols) in drought-stressed plants.

An increase in phenolic, proline, and flavonoid content was also shown in ornamental plants (*Spiraea nipponica* and *Pittosporum eugenioides*) subjected to mild drought stress condition [139]. Citrus sinensis L. drought-stressed improved water use efficiency when treated with extracts of A. nodosum (Spann et al., 2011) [140].

Some other examples are summarized in Table 5.

**Table 5.** Drought stress physiological and biochemical changes in agriculture crops treated with seaweed extracts.

| Seaweed Extracts Description | Crop | Growing Conditions | Drought Stress Treatment | Plant Growth Stage at the Stress Treatment Application | Effects of Protein Hydrolysates on Stressed Crops | References |
|---|---|---|---|---|---|---|
| *Ascophyllum nodosum*/ Foliar spray | *Solanum lycopersicum* (cv. Moneymaker) | Growth into pots placed in a growth room and filled with vermiculite/perlite and slow releaser fertilizer | Interruption of watering for 7 days | 35-day-old tomato plants | Increase in: RWC, plant growth, foliar area, chlorophyll, proline, soluble sugars<br>Decrease in lipid peroxidation | [133] |
| *Ascophyllum nodosum*/aminoacidic Soil application/ foliar spray | *Brassica oleracea* var. italica | Growth into pots placed in a growth chamber and filled with peat and complex fertilizers | Interruption of watering for 2 days | Seven weeks after planting | Increase in photosynthesis, stomatal conductance, and chlorophyll content | [141] |
| *Ascophyllum nodosum*/ foliar and drench | Spinach (cv. Bloomsdale) | Growth into pots filled with sand and topsoil, placed in a growth chamber | 100% (full irrigation) and 50% (drought stress) evapotranspiration | 3 weeks after sowing | Increase in plant growth, leaf relative water content, area, fresh weight, dry weight, and specific leaf area, improvement of gas exchange parameters<br>Decrease in ferrous ion chelating ability | [132] |
| *Ascophyllum nodosum*/ Foliar application | *Saccharum* spp. | Field | driest period of the year | late-harvest sugarcane | Increase in biomass production and stalk yield, sugar yield, antioxidant enzyme activity, and cellular redox balance<br>Decrease in malondialdehyde content | [136] |

**Table 5.** *Cont.*

| Seaweed Extracts Description | Crop | Growing Conditions | Drought Stress Treatment | Plant Growth Stage at the Stress Treatment Application | Effects of Protein Hydrolysates on Stressed Crops | References |
| --- | --- | --- | --- | --- | --- | --- |
| Extracts from *Fucus spiralis*, *Ulva lactuca*, *Laminaria ochroleuca*, and *Ascophyllum nodosum*/soil drench and foliar spray | *Vicia faba* (cv. Super Aguadulce) | Growth into pots filled with natural soil and placed in a covered shelter | Water withholding for 10 days | 40 days after sowing | Increase in plant biomass, relative water content, proline content, and soluble sugars content<br>Decrease in malondialdehyde content | [142] |
| *Ascophyllum nodosum* | Soybean | Growth into pots irrigated with nutrient solution and placed in a growth chamber | 75 h of water interruption | 14 days after transplanting | Increase in relative water content, stomatal conductance, and antioxidant activity | [143] |
| *Ecklonia maxima* | *Chicorium intybus* | Growth into pots filled with peat and placed in a greenhouse | Moderate (60–70% of water holding capacity) and severe (30–40% water holding capacity) stress | 7 days after transplanting | Increase in fresh biomass, relative water content, water use efficiency, nitrogen use efficiency, P, K, Ca and Mg content, chlorophyll content, proline and total polyphenols content<br>Decrease in plant growth traits and yield and N content | [140,144] |

### *4.3. Genes Involved in Drought Tolerance in Agriculture Crops Treated with Seaweed Extracts*

Seaweed extracts have been found to increase chalcone isomerase, the plant phenylpropanoid precursor enzyme involved in plant defense against stress [102].

*A. nodosum* extract was found to increase the gene expression encoding the nitrate and auxin transporter NRT1.1. in *Arabidopsis thaliana*. In this way, the extract caused an increase in the growth of lateral roots and the assimilation of nitrate [145]. Furthermore, commercial *A. nodosum* extract was found to increase the expression of the NodC rhizobial bacterial gene. This gene is involved in the rhizobia-plant interaction and the induction of root nodule formation. Therefore, in the presence of the extract, leguminous plants had a greater number of nodules and fixed more nitrogen [146]. Extracts from a commercial brown algae extract increased the expression of genes encoding enzymes regulating nitrogen metabolism, antioxidant activity, and glycine betaine synthesis in treated spinach plants. The increase in these enzymes was associated with an increase in phenolic compounds, total soluble proteins, and the antioxidant capacity of plants [127].

According to Goñi et al. [133], changes in the expression of tas14 dehydrin gene were responsible for the increased tolerance of tomato plants subjected to drought. This gene encodes phosphorylated proteins that accumulate during drought stress. Shukla et al. [143] attributed the increased drought tolerance of soybean plants to the increased activity of the genes GmCYP707A1a, GmCYP707A3b, GmRD22, GmRD20, GmDREB1B, GmERD1, GmNFYA3, FIB1a, GmPIP1b, GmGST, GmBIP and GmTp55. These genes are involved in the synthesis and regulation of abscisic acid levels, photoprotection against photoinhibition, and the synthesis of aquaporins.

Biostimulant Super Fifty obtained from *Ascophyllum nodosum* repressed the stress-responsive negative growth regulator (RD26) in *Arabidopsis thaliana* plants subjected to drought stress. In this way, the plants had an active cell cycle during stress. Furthermore, stressed plants treated with the biostimulant increased the expression of CYCP2;1, a gene that promotes meristem cell division [147].

## 5. Silicon

### *5.1. Origin and Effectiveness of Silicon as Biostimulants in Agriculture*

Silicon (Si) is a pervasive constituent of soil fractions, encompassing both solid and liquid phases, where its interactions play pivotal roles in soil physicochemical dynamics. Within the liquid phase, Si exists predominantly in dissolved form, comprising monosilicic and polysilicic acids, alongside an array of complexes formed with inorganic, organic, and organosilicon compounds. The presence of Si in soil solution underscores its intricate involvement in soil biogeochemical processes and highlights the importance of elucidating its behavior and fate within soil matrices. Such understanding is fundamental to advancing our comprehension of soil Si cycling and its implications for ecosystem functioning and agricultural productivity [148–151].

Silicon (Si), which is the second most abundant element in the Earth's crust, is considered nonessential for plant growth and development. In any case, its importance lies in its multifaceted role in promoting various physiological processes in plant organisms. The concentration of Si in soils in complex forms such as aluminum and crystalline silicates exhibits considerable variability, ranging from 1% to 45%, depending on the type of soil. In addition, the classification of Si as a macro- or micronutrient in plant tissues depends on its concentration relative to dry weight. In this regard, silicon is considered a macroelement when it is present in amounts above 0.1 percent of dry weight, while it assumes the classification of micronutrients when concentrations fall below 0.05 percent of dry weight. This categorization underscores the contextual significance of silicon in crop physiology, the importance of which varies depending on plant species and environmental contexts and their mutual interaction [151–155]. Silicon-based products represent a spectrum of formulations, encompassing both solid and liquid states. Solid silicon products are derived from a variety of sources, including geological formations such as rocks and sediments, by-products originating from plant materials, as well as recycled materials. Consequently, the silicon content and properties of these solid formulations exhibit significant variability, contingent upon the compositional attributes of the respective raw materials employed. This diversity underscores the nuanced interplay between raw material characteristics and the resultant attributes of solid silicon products, thereby influencing their efficacy and suitability for facilitating plant uptake [156]. Liquid formulations encompass a spectrum of compositions, including monosilicic or polysilicic acid solutions. The silicon concentration within liquid formulations directly dictates the available silicon content accessible to plants. Notably, products with elevated silicon concentrations tend to exhibit alkaline pH levels, typically around 9, necessitating dilution to preempt potential soil pH perturbations upon application. Furthermore, colloidal gels comprising silicic acid offer an additional modality for silicon formulation, presenting opportunities for nuanced delivery strategies within agricultural paradigms [153,157].

Silicon products offer versatile application methods, mainly through soil incorporation or foliar application. Among these methods, soil application stands out as the most effective strategy for increasing silicon concentration in plant tissues due to its effectiveness in facilitating silicon uptake. At the time of application, silicon is mainly absorbed in the form of silicic acid at the root level. Subsequently, facilitated by xylem vessels, silicon is transported throughout the plant via the transpiration stream. At transpiration sites, Si tends to accumulate predominantly in the form of amorphous silica, showing a characteristic pattern of localization near anatomical elements such as stomatal openings, trichomes, lumens, and intercellular voids. This spatial distribution reflects a preferential deposition of Si in regions intricately involved in water regulation and gas exchange, indicating a functional correlation between Si localization and the physiological processes occurring at these sites [156,158]. Foliar application, although less efficient than soil incorporation, remains a viable strategy for increasing the concentration of Si in plant tissues [157,159]. Effective foliar application generally requires the use of high concentration sprayed solutions, reaching levels as high as 1500 ppm. Despite its lower efficiency, foliar application offers significant advantages, particularly in circumventing potential problems associated with immobilization of Si in soil. As a result, it is often favored in scenarios that require repeated sprays targeting specific plant organs. In the context of foliar uptake, silicon can be absorbed directly through the cuticular layer or through various openings on the leaf surface, including clefts adjacent to trichomes, stomata, pores and hydathodes. This mode of uptake underscores the versatility of foliar application in facilitating Si uptake, highlighting its utility in strategies for targeted Si incorporation into agricultural systems [157,159].

### *5.2. Morphological, Physiological, and Biochemical Changes Induced from Silicon to Mitigate Drought Stress in Agriculture Crops*

In addition to its established function as a vital plant mineral nutrient, Si has gained attention as a biostimulant due to its ability to modulate a plethora of plant biochemical and physiological processes. Beyond its conventional role in nutrient uptake, silicon demonstrates multifaceted effects that result in pronounced improvements in plant growth, photosynthetic efficiency, and resilience to environmental stressors. These effects may result from mechanical and/or metabolic alterations that occur in Si-treated plants [160,161]. Mechanical changes are commonly attributed to the deposition of silica, leading to the formation of phytoliths within the cell walls of epidermal cells [162]. Phytolith deposition leads to an augmentation in cell wall thickness and mechanical strength, yielding several advantageous outcomes for plant physiology and resilience. Specifically, the enhanced structural integrity afforded by phytoliths positively impacts leaf orientation, thereby promoting optimal positioning for photosynthetic efficiency. Furthermore, the reinforced cell walls bolster overall plant sturdiness, providing robust defense mechanisms against diverse environmental threats, whether biotic or abiotic in nature [163,164].

Metabolic alterations induced by Si applications are critical in mitigating ROS-induced oxidative damage in plants facing various stresses. Si has been shown to enhance the antioxidant activity of specific enzymes, particularly superoxide dismutase (SOD), catalase (CAT), and ascorbate peroxidase (APX). This increase in antioxidant enzyme activity serves to safeguard plant cells from the damaging effects of ROS, thereby preventing the degradation of essential biomolecules such as proteins, lipids, carbohydrates, and DNA. By strengthening antioxidant defenses, silicon confers resilience to oxidative stress, thereby supporting plant vigor and adaptability under adverse environmental conditions [165,166].

In addition to its role in mitigating oxidative damage, Si has been observed to increase water use efficiency (WUE) under drought conditions by attenuating cuticular and stomatal water losses associated with transpiration. This effect is mediated by Si-induced changes in the structural and physiological attributes of plant surfaces. Specifically, Si treatments lead to alterations in cuticular properties and stomatal behavior, resulting in reduced rates of water loss by transpiration. By reducing transpiration water loss through these mechanisms, silicon supplementation contributes to WUE optimization, thereby enhancing plant resilience to drought stress [151]. The most common Si formulation tested in these experiments was sodium metasilicate ($Na_2SiO_3$) and monosilicic acid ($H_4SiO_4$), applied via foliar in field or under greenhouse conditions. The dosage was dependent on the experimental design tested in each trial (Table 6).

Rahimi et al. [167] investigated the ameliorative effects of the application of selenium in *Calendula officinalis* subjected to drought stress conditions. Drought stress was simulated using Polyethylene glycol (PEG) at different levels, including 0 (control), −0.5 (mild), −1 (moderate), and −1.5 MPa (severe stress). Drought stress was applied two weeks after germination. The silicon treatments involved the application of silicon nanoparticles (SiNPs) at concentrations of 0, 100, 200, 500 mg $L^{-1}$), as well as silicate at concentrations of 0, 1, 1.5, 2 mg $L^{-1}$) supplied via seed priming. The results revealed that the treatments significantly enhanced the germination rate and index in seedlings subjected to drought stress. Ning et al. [168] examined the effect of drought stress on *Zea mays* cultivated in pots. Drought stress was applied at the 6-leaf (D-V6), 12-leaf (D-V12), and blister (D-R2) growth stages, consisting of moderate drought stress (50% field capacity) for a duration of 7 days. To ameliorate the damage caused by drought stress, silicon fertilizer was supplied as $Na_2SiO_3.9H_2O$ at two levels: 0 (-Si) and 0.06 mg Si $kg^{-1}$ dry soil (+Si). The findings obtained revealed that silicon application enhanced leaf area, photosynthetic rate and SOD, POD and CAT activities in maize plants.

Additional results are summarized in Table 6.

**Table 6.** Drought stress physiological and biochemical changes in agriculture crops treated with silicon.

| Silicon Description | Crop | Growing Conditions | Drought Stress Treatment | Plant Growth Stage at the Stress Treatment Application | Effects of Silicon on Stressed Crops | References |
|---|---|---|---|---|---|---|
| Monosilicic acid ($H_4SiO_4$) with 20% Si. A total of 0, 0.75, 1.5 and 3 g SA (corresponding to 0, 100, 200 and 400 kg SA $ha^{-1}$, respectively) was applied in each pot at an interval of 2 weeks | *Cucumis melo* L. cv. Cantaloupe | Potted plants under polyhouse conditions | Three soil moisture regimes (100%, 75% and 50% FC) | From vegetative until fruit maturity | Increased fruit yield and total soluble solids (°Brix) | [169] |
| Foliar application of $K_2Si_2O_5$ at rates of 1, 2 and 3% | *Triticum aestivum* L. | Field conditions | Irrigation regimes: control, irrigation stopped at 30 days after sowing (DAS) (vegetative stage), and irrigation stopped at 75 DAS (reproductive stage) | From vegetative to harvesting | Increased number of spikes per plant, 1000-grain weight and grain yield | [170] |
| Sodium metasilicate ($Na_2SiO_3$) (2 mM) drenched in the soil of the pots (500 mL $kg^{-1}$) | *Lens culinaris* | Potted plants | Control (C) with 100% field capacity (FC), moderate stress (MD), 50% FC, and severe stress (SD), 20% FC. | Onset of flowering period for 28 days | Increased APX, GPX, CAT, SOD, GR, DHAR and nitrate reductase activities | [171] |
| Foliar application of monosilicic acid ($H_4SiO_4$) at 0, 2000 and 4000 ppm | *Cucurbita pepo* L. | Field conditions | Two levels of water irrigation at 80% of water holding capacity (WHC) as control and 50% of WHC as drought treatment | Sprayed at three times with 10 days intervals starting at the 10th day from transplanting until 40 days from transplanting (DAT) | Increased chlorophylls concentration, SOD, CAT, POD and polyphenol oxidase (PPO) activities | [172] |

**Table 6.** *Cont.*

| Silicon Description | Crop | Growing Conditions | Drought Stress Treatment | Plant Growth Stage at the Stress Treatment Application | Effects of Silicon on Stressed Crops | References |
|---|---|---|---|---|---|---|
| Calcium metasilicate ($CaO.SiO_2$) at levels of 0, 20, 40, 60, 80 and 100 g $CaO.SiO_2$ $pot^{-1}$ | *Saccharum* spp. | Potted plants under greenhouse conditions | Drought stress [mild −75 ± 5, moderate—50 ± 5 and severe −25 ± 5% of soil water content capacity (SWCC)] | During the drying cycle (up to 122 days). | Enhanced plant growth and photosynthetic pigments as well as SOD, CAT and GPX activities | [173] |
| Foliar spray ($K_2SiO_3$) (Sifol®) | *Vigna unguiculata* L. | Field conditions | 100% evapotranspiration replacement (W100) and 50% evapotranspiration replacement (W50) | Cowpea phenological (stages V5 and V9) | Increased leaf proline concentration and APX and CAT activities | [174] |
| Silica sol and choline-stabilized orthosilicic acid (ch-OSA) directly applied in the nutrient solution | *Tagetes patula* L. | Hydroponic under greenhouse conditions | Drought stress for 55 h | Application of silica soillevel I (23.25), level II (31.0); ch-OSA (mg $dm^{-3}$ of nutrient solution NS) | Increased net photosynthesis activity (PN), stomatal conductance (gs), and transpiration rate (E) | [175] |
| Foliar application Si 1.5 mM (10 mL per pot) | *Oryza sativa* L. | Puddled earthen pots | Two moisture regimes (100% and 40% water holding capacity) from 15th day of transplantation to 30th day of transplantation | Interval of ten days during the drought period | Enhanced plant growth and development, yield and quality traits | [176] |
| Foliar application of 0, 0.5, 1, and 1.5 mM nano-$SiO_2$ | *Vicia faba* L. | Field | Two moisture regimes (100% and 65% ETc) | From flowering to maturity | Enhanced leaf gas exchange, water relations and nutrient uptake | [177] |

### 5.3. Genes Involved in Drought Tolerance in Agriculture Crops Treated with Silicon

The use of Si in agricultural practices has been shown to cause changes in gene expression in crop species. These alterations in gene expression induce a myriad of physiological and biochemical changes, collectively increasing plant growth, enhancing stress resistance and overall yield potential [178,179]. Although extensive literature elucidates systemic physiological and metabolic changes in drought stress-exposed and Si-treated crops, a significant gap in transcriptomic investigations persists. This dearth underscores the imperative for expanded research initiatives aimed at elucidating the regulatory roles of genes in orchestrating plant responses to stressors. By leveraging transcriptomic analyses, researchers can unravel the intricate molecular pathways underpinning stress tolerance mechanisms, thus advancing our comprehension of plant stress physiology at the genetic level. This concerted effort holds a significant promise for informing targeted strategies to enhance crop resilience and mitigate yield losses under challenging environmental conditions. In this exhaustive review, we have meticulously synthesized findings from the existing literature, collating a comprehensive array of references pertaining to genes intricately involved in fundamental physiological processes. Specifically, our review encompasses genes associated with photosynthesis, amino acid synthesis, photorespiration, and membrane proteins. A concise presentation of these referenced genes is provided in Table 7.

**Table 7.** Drought stress responsive genes in agriculture crops treated with silicon.

| Biostimulant | Crop | Growing Conditions | Drought Stress Treatment | Genes Activated by Drought Stress | References |
|---|---|---|---|---|---|
| Addition of potassium silicate ($K_2SiO_3 \cdot nH_2O$) 2.5 mM in the nutrient solution | Tomato | Hydroponic | 10% PEG-6000 from 7 days | Downregulation of *PetE*, *PetF*, *PsbP*, *PsbQ*, *PsbW*, and *Psb28* under water stress | [180] |
| Addition of monosilicic acid (0.75 mM) in the nutrient solution | Tomato | Hydroponic | 1% PEG-6000 in the nutrient solution for 21 days | Increased relative expression of argininosuccinate lyase (SlASL) | [181] |
| Addition of 0.6 mM Si in the form of $Na_2SiO_3$ in the nutrient solution | Tomato | Hydroponic | 1% PEG-6000 from the emergence of two true leaves until the emergence of four true leaves | Reduced leaf expression levels of Aox1a, Aox1b, and Aox1c under water scarcity | [182] |
| Addition of potassium silicate ($K_2SiO_3 \cdot nH_2O$) 2 mM in the nutrient solution | Sorghum | Greenhouse in soil | Water field capacity (−18 KPa) until water deficiency (−138 KPa) for 17 days | Increased root expression levels of *TIP4;2* and *PIP1;3/1;4* and reduced root expression level of *PIP1;6* under water stress | [183] |

## 6. Conclusions

The biostimulants ability to enhance soil health, promote nutrient uptake, and mitigate drought stress renders them highly attractive in the pursuit of sustainable, climate-resilient agriculture. The scientific community has placed significant emphasis on biostimulants due to their potential to enhance plant growth and resilience, particularly under stressful conditions such as drought.

Several promising areas warrant further investigation in future research on the role of biostimulants as alleviators of drought stress in plants. For instance, conducting long-term, multi-season field trials is crucial to assess the sustained efficacy of biostimulants and to address the poor lab-to-field translation, as well as the lack of robustness across varying climatic conditions. Moreover, the optimization of biostimulant formulations, alongside precise tailoring of their timing and dosage, should be adapted to specific crops, soil types, and environmental conditions to maximize drought-mitigation potential. Emphasizing the agroecological perspective of these products through a range of field experiments, particularly in the contexts of organic farming, agroforestry, and regenerative agriculture practices is essential. Such studies are key to developing resilient agricultural systems in drought-prone regions, ensuring that biostimulants align with sustainable farming principles and contribute to long-term environmental and agricultural sustainability.

In any case, it should be made clear that the application of biostimulants, regardless of their origin, will not be able to replace synthetic fertilizers but could help reduce their use by improving the sustainability of agricultural production.

**Author Contributions:** P.G.-C. and M.G.; writing—original draft preparation, P.G.-C., M.C., Y.R. and M.G. writing—review and editing. All authors have read and agreed to the published version of the manuscript.

**Funding:** This research received no external funding.

**Data Availability Statement:** The data are contained within the article.

**Conflicts of Interest:** The authors declare no conflicts of interest.

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