Amino Acids Biostimulants and Protein Hydrolysates

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The effects of different types of biostimulants on crops include improving the visual quality of the final products, stimulating the immune systems of plants, inducing the biosynthesis of plant defensive biomolecules, removing heavy metals from contaminated soil, improving crop performance, reducing leaching, improving root development and seed germination, inducing tolerance to abiotic and biotic stressors, promoting crop establishment and increasing nutrient-use efficiency. Protein hydrolysates are mixtures of polypeptides and free amino acids resulting from enzymatic and chemical hydrolysis of agro-industrial protein by-products obtained from animal or plant origins, and they are able to alleviate environmental stress effects, improve growth, and promote crop productivity. Amino acids involve various advantages such as increased yield and yield components, increased nutrient assimilation and stress tolerance, and improved yield components and quality characteristics.

amino acids biostimulants medicinal plants phenols protein hydrolysates

1. Introduction

Biostimulants are considered bioactive substances that are either inorganic or organic microorganisms that can increase crop performance when utilized in small quantities [1] as they can enhance both performance and growth as well as improve nutrient- and water-use efficiencies of different crops [2][3][4][5][6][7][8]. Amino acids have a dual function as building blocks for proteins and as providers of organic nitrogen, which can alleviate the negative impacts of drought and salt stress [9], and promote cell growth. They are vital in metabolite synthesis, growth, and development, and appropriate in plants because of their structure as protein units [10][11][12][13][14]. The positive effects of the foliar application of amino acids and biostimulants based on amino acids on both the qualitative and quantitative characteristics of *Foeniculum vulgare* Mill, *Coriandrum sativum* L., *Achillea millefolium* L., *Nigella sativa* L., *Ocimum basilicum* L., *Urtica pilulifera* L., *Mentha piperita*, *Calendula officinalis* L., and *Satureja hortensis* L. plants have been reported [11][12][13][14][15][16][17][18][19][20][21][22][23][24][25].

Amino acids used for the production of biostimulants are obtained from the chemical synthesis of plant proteins, such as algae, soybean, and corn, as well as from animal proteins by both chemical and enzymatic hydrolysis. Amino acids that have been used for foliar usage are the result of enzymatic hydrolysis from both animal and plant protein hydrolysates, and as it is very energy-consuming, foliar application is a common process in the agricultural industry. Protein hydrolysate is related to the product of the hydrolytic action of protease(s) on a pure protein sample, or a complicated proteinaceous sample [26][27], which is necessarily a mixture of peptides, free amino acids, and probably partially degraded proteins [28][29]. Protein hydrolysates and amino acids, which are also known

as protein-based biostimulants, are usually readily available because of the abundance of raw materials and their affordable cost [30][31][32]. Protein-based biostimulants can usually be obtained from the hydrolysis of protein-rich agro-wastes, which includes chemical, thermal, and enzymatic processes, or a combination of them [33][34][35][36]. They are usually considered as a crude peptide mixture, and they are usually used as the initial raw material for bioactivity testing [37][38][39]. Fish protein hydrolysates are famous in different parts of the world for pharmaceutical, cosmetic, and nutritional usage [40][41][42].

2. Amino Acids

Amino acids for the production of biostimulants are derived by chemical synthesis from plant proteins such as soybean, corn, algae, corn, etc., as well as from animal proteins by enzymatic and chemical hydrolysis [43][44][45][46][47][48]. Amino acids act as vital molecules with various physiological roles [49] and play an important function in seed germination [50][51], and under salinity stress, they can behave as osmolytes, which can promote stomatal opening control, transport regulation, enzyme activation, heavy metals detoxification, redox homeostasis maintenance, and gene expression [52][53][54][55][56]. Supplementing plants with environmentally friendly amino acid biostimulants can decrease the application of inorganic fertilizers [57][58].

Amino acids are also important in the agriculture industry as chelates of metal ions and microelements chelated with amino acids from very small, electrically neutral molecules increase their transport and absorption within the plant [59][60][61]. Some of the most important products in the market which contain amino acids are Delfan Plus (Tradecorp, Madrid, Spain), Natural Crop SL (Natural Crop Poland Sp. Z o.o., Warsaw, Poland), Bosfoliar Activ (COMPO EXPERT, Munster, Germany), Amino Quelant Ca (Bioiberica, Barcelona, Spain), Tecamin Max, Tecamin Brix, Tecnokel Amino Mix, Terra-Sorb Foliar (Agritecno Fertilizants, Valencia, Spain), Agrocean B (Agrimer, Plouguerneau, France), Metalosate Calcium and Metalosate Fe (Albion Minerals, Layton, UT, USA) [62][63][64][65]. The usage of amino acids can increase co-enzyme formation and the photosynthesis procedure [66], and supports different plant organisms that may face environmental stresses [67]. It has been also reported that the exogenous utilization of amino acids can enhance nitrogen status, and the contents of mineral elements in plant tissues [68][69]. Depending on environmental conditions and plant species, plants reduce inorganic nitrogen to amino acids in roots, nodules, and leaves [70][71][72]. Many studies have reported the important and notable effects of the foliar application of concentrations with phenylalanine and tyrosine solutions on essential oil, the total amount of phenols, and their compositions in *Ocimum basilicum* L., *Melissa officinalis* L., and *Coleus blumei* L. plants [73][74][75]. Phenylalanine is an amino acid [76][77], and its foliar application can help mustard (*Brassica campestris* L.) plants overcome drought stress and increase total chlorophyll contents, shoot length, and biological yield [78]. Roman et al. [79] reported that foliar application of methyl jasmonate and phenylalanine can increase the content of volatile compounds in grapes, and Portu et al. [80] introduced it as an important management tool for boosting grape quality. The main mechanisms of amino acids biostimulants are shown in **Figure 1**.

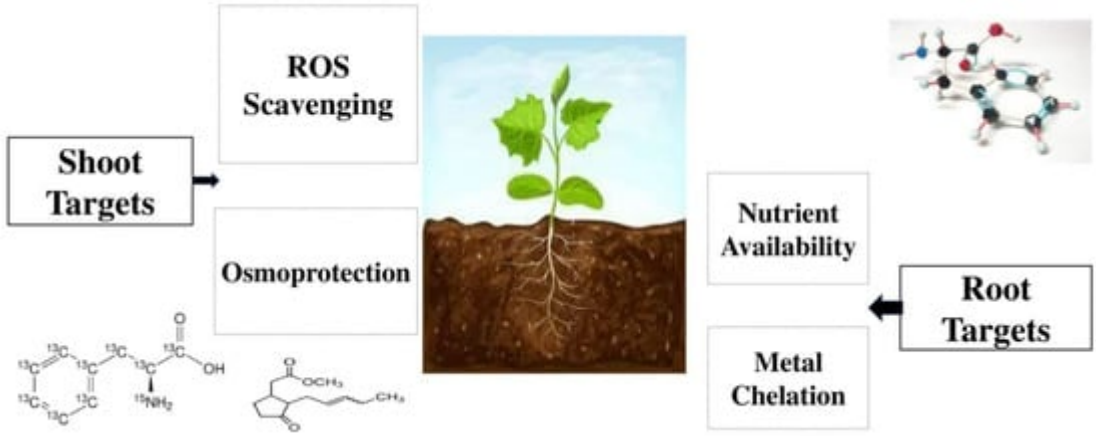

Figure 1. The most important mechanisms of amino acids biostimulants.

3. Protein Hydrolysates

Protein hydrolysates, especially those that contain antioxidant peptides, are obtained from natural components, and many researchers and scholars consider them biostimulants because of their minimum side effects, easy absorption, low cost, high activity, and lower molecular weight [81][82][83][84][85][86][87][88]. Protein hydrolysates and peptides can be used as notable ingredients in the formulation of functional foods [89][90][91][92][93][94][95][96][97][98][99]. They can be used as foliar sprays or through drip irrigation systems, and the amino acids can be absorbed through both leaves and roots [100][101][102]. Their utilization can significantly affect nitrogen metabolism in plants, and boost productivity, particularly when applied as a seed pre-treatment [102]. For separating the amino acids in protein hydrolysates, a liquid chromatography process can be used [103][104]. Numerous methods have been considered to produce hydrolysates from fish and fish by-products such as thermal hydrolysis, autolysis, chemical hydrolysis, and enzymatic hydrolysis [104][105]. The basic procedures utilized following hydrolysis of protein are heat inactivation, which has a function in the inactivation of proteolytic enzymes; ultrafiltration, which is important in the removal of high molecular weight peptides and proteins; use of specific enzymes, which can reduce the content of specific amino acids; hydrolysis by exoproteases, which is active in hydrolysis and the reduction of bitterness; carob activation, which has a notable role in the reduction of bitterness; and absorption chromatography, which can decrease the content of aromatic amino acids. Microbial-based biostimulants such as Environoc 401®, Bioyield®, Rootshield Plus+ WP ®, Spectrum + Myco®, Select®, and Endomaxx® inconsistently increased the quality of bell pepper (*Capsicum annuum* L.) in a greenhouse experiment [106]. Ghorbel-Bellaaj et al. [107] reported that five proteolytic enzymes, namely Alcalase®, trypsin, a crude enzyme extract from sardinelle (*Sardinella aurita*) viscera, and an enzyme preparation from *Aspergillus clavatus* ESA and *Bacillus licheniformis* NH1, which are protein hydrolysates, were obtained from shrimp via by-products processing, and they have revealed notable degrees of antioxidant activities, such as β-carotene bleaching, reducing power, and 1,1-diphenyl-2-picrylhydrazyl (DPPH)-scavenging activity assays, which can be a promising and helpful alternative for accessible commercial nitrogen sources from other origins. It can be a good source for microbial growth and protease production by *Saccharomyces cerevisiae*, *Escherichia coli*, *Bacillus subtilis* A26, and *Bacillus mojavensis* A21.

Some of the available plant biostimulants, their composition, and application strategies are C Fish, which contain peptides and amino acids that are used on vegetables and fruits to increase the plant's resistance to insect pressure, disease and drought or heat stress which originates from white fish/mixed fish composition autolysates and hydrolysates in fruits and vegetables; Radifarm, which contains peptides, amino acids, betaines, saponins, vitamins, polysaccharides, and microelements, has been used to promote the formation of an extensive root system by speeding up the elongation of adventitious and lateral roots of vegetables and fruits; Megafol, which contains betaines, amino acids, auxin, vitamins, proteins, cytokine, and gibberellin, can improve the balance between vegetative productivity and development as well as plant resistance to stressors such as hail, weeding, root asphyxia, and frost; Biozyme, which includes plant hormones, algae extract, and chelated micronutrients, can boost nutrient uptake, photosynthesis, and the activity of chlorophyll of legumes, vegetables and fruits; BioRoot, which contains humates, plant and mineral-derived organic acids, enhances rooting ability, protein content, and chlorophyll of fruits and vegetables; Grow-plex SP, which contain humic acids, can increase soil bacteria, shoot and root growth, and zinc and iron uptake of vegetables and fruits; Ergonfil, which has cysteine, animal protein hydrolysates, keratin derivatives, and folic acid, can promote chlorophyll synthesis and indole acetic acid, increase chelation, and improve translocation in fruits and vegetables; Benefit, which contains nucleotides, amino acids, vitamins, free enzymatic proteins, can improve cell division and increase the number of cells per fruit [108][109][110][111]. Animal-derived gelatin, which has peptides and amino acids, can improve shoot dry weight and promote root nitrogen assimilation in broccoli, arugula, tomato, pepper, cucumber, and field corn [112]. There are notable reports and evidence that the application of non-structural and structural amino acids, such as histidine, proline, taurine, and glutamate, can provide protection to the plant from environmental stresses or play an important function in metabolic signaling by regulating nitrogen acquisition by the roots [113][114]. Amino acids can act as osmoprotectants, which stabilize membranes, enzymes, and proteins against denaturing caused by high salt components and non-physiological temperatures [115]; moreover, arginine has been proven to have an important function in nitrogen transport and storage in plants during biotic and abiotic stress conditions [116]. Amino acids can also reduce plant toxicity by heavy metals by acting as metal chelators [117][118]. Rouphael et al. [119] reported that the application of vegetal-protein hydrolysates based microgranules can increase carotenoids and total chlorophyll content. Protein hydrolysate has a positive influence on total root area and on root length, which can increase mineral-nutrient and water-use efficiency as well as promote plant productivity and resistance to harmful conditions [120][121][122]. It can also positively influence the leaf area and yield of horticultural plants and fruit trees [123][124]. The exogenous utilization of protein hydrolysate and isolated amino acids can promote plant antioxidant performance by improving the non-enzymatic and enzymatic antioxidant defense machinery of the cell [125].

4. Phenols and Phenolic Biostimulants

Phenols are a major type of antioxidant phytochemical, which have significant importance because of their free radical scavenging and biological characteristics [126][127][128][129]. Phenolic compounds are the most abundant secondary metabolites in many plants which are usually discovered in the cell walls of subepidermal and in the vacuoles of epidermal cells [130][131]. Endogenous phenolic components in plants have different functions, which can be used by plants to defend themselves against pathogens, herbivores, and weeds. They are implicated in

seed germination and dormancy, appropriate as screens against damaging UV radiation, and act as pigments to attract seed dispersal agents and pollinators [132][133][134]. The function of phenolic acids as signaling molecules in plant-microbe symbioses has been reported in previous research [135]. Some of the most important phenolic compounds with bioprotectant activities are ferulic acid, curcumin, ellagic acid, catechol, gallic acid, coumarin, caffeic acid, catechin, quercetin, sinapic acid, rutin, resveratrol, salicylic acid, and syringic acid [136][137]. The accumulation of phenolic compounds and the subsequent production of quinones in turnip (*Brassica rapa* L.) may happen when plants are susceptible to Boron deficiency [138]. Phenolic compound concentration can be important in the biochemical pathway of toxigenic fungal species because of the induction of stress via sub-lethal contents and depletion of the phenolic compounds [139]. Phenolics have meaningful functions in plant development, especially in pigment and lignin biosynthesis as well as considerable roles in plant protection against stress. It has been reported the correlation between antifungal activity and total phenolics of plants [140] and the accumulation of amino acids and phenolics may boost tolerance to both copper and cobalt stress in barley [141]. Silva et al. [142] reported that tyrosol, which is a phenolic compound from olive oil and several endophytic fungi such as *Phomopsis* sp., can be used as an important biostimulant in soybean seed treatment, which can alter soybean plant metabolism without meaningful impacts on crop yield. Masondo et al. [143] reported that two phenolic biostimulants, namely eckol and phloroglucinol, isolated from brown algae *Ecklonia maxima* can have a significant effect on the phytochemical and growth of *Eucomis autumnalis*. While the phenolic acid metabolism in *Kandelia obovata* may decrease the negative impacts of cadmium and zinc [144], it has been reported that the phenolic compounds of leave extracts of *Calligonum arich* L. are effectual against pathogenic bacteria [145], and the phenolic compounds of apricot branches have shown antifungal activity against *Monilinia laxa* growth [146][147][148]. One of the notable impacts of phenolics is to improve the resistance of *Nicotiana langsdorffii* to Cr(VI) [149][150][151][152].

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