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**From Waste to Wonder: The Potential of Protein Hydrolysates as Plant Biostimulants in  
Agriculture**

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**Abstract**

Protein hydrolysates (PHs) derived from plant and animal byproducts have emerged as promising biostimulants in sustainable agriculture. This comprehensive review investigates the production, composition, and mechanisms of action of PHs in promoting plant growth and stress resilience. PHs are obtained through enzymatic hydrolysis of protein-rich waste, yielding mixtures of amino acids and bioactive peptides. Commercial PHs exhibit diverse effects, including enhanced nutrient uptake, abiotic stress tolerance, and crop yield in various

agricultural and horticultural species. The biostimulant activity of PHs is attributed to multiple interconnected pathways, such as hormone-like signaling, antioxidant defense, osmotic regulation, and gene expression modulation. Moreover, PHs influence the plant microbiome, potentially contributing to improved stress adaptation. Foliar and root application methods, along with factors like pH and transporters, govern PH absorption and translocation in plants. Transcriptomic and proteomic studies have provided insights into the molecular mechanisms underlying PH-mediated growth promotion and stress mitigation. Future research should focus on optimizing PH formulations, exploring synergies with other biostimulants, and elucidating the role of plant-microbe interactions. By harnessing the potential of these sustainable compounds, PH biostimulants offer a promising approach to enhance agricultural productivity and resilience in the face of global challenges.

**Keywords:** Protein hydrolysate, Biostimulant, Valorization, Byproducts, Abiotic stress, Sustainable agriculture

## 1. Introduction

The global agricultural industry is progressively adopting sustainable methods, with a focus on preserving and rejuvenating vital habitats, enhancing soil wellness, and ensuring the quality of water resources (MacLaren et al., 2022). This vision represents a truly transformative change driven by the integration of sustainable agriculture into government policies and growing consumer demand. For centuries, farming practices have increasingly turned to synthetic inputs to maximise crop yields and safeguard against pests, without fully accounting for the detrimental consequences on the environment and the long-term health of upcoming generations (Buffagni et al., 2021). Currently, one of the primary objectives of agriculture is to

implement eco-friendly production techniques that prioritise long-term sustainability and production of high-standard food (Ceccarelli et al., 2021a). This trend has contributed to the expansion of the agrosience market, in which biostimulants are the leading flagship products (Ebinezzer et al., 2020). Biostimulant application is experiencing rapid growth, with its implementation covering an extent of 24.9 million hectares until 2021 for plant growth (Jagadeesan et al., 2024). However, efficacy studies of biostimulants are challenging because of their complexity and cannot be attributed solely to a single component (Gupta et al., 2022). PH is a promising category of biostimulants that vary in the type of amino acids, oligopeptides, and polypeptides depending on the raw material used and the method of hydrolysate preparation. The agronomic perspective of PH-based biostimulants is to increase crop yield, which is frequently proportional to the enhanced absorption of carbon, nitrogen, and sulphur, thereby improving photosynthesis and increasing the levels of free proteins, carbohydrates, amino acids, pigments, phenolic compounds, and enzymes (Ciriello et al., 2024). PHs can serve as primary sources of amino acids for the biosynthesis of complex molecules, thereby promoting cell growth and protecting cells from deformation (Flaibam et al., 2024). A rich blend of amino acids and peptides is a readily available source of nitrogen, which is directly used by plants as a nutrient source (Antón-Herrero et al., 2023). The growing population and increasing industrialisation have led to the production of large quantities of organic wastes. These waste materials are classified into several categories, including slaughterhouses, dairy waste, households, and municipal, agricultural, and industrial wastes. On a global scale, approximately 100 million tons (Mt) of waste rich in protein is produced each year (Ling et al., 2022)(Zhao et al., 2022). According to the EPA (Environmental Protection Agency) in 2015, household garbage landfills produced emissions equal to 115.7 Mt of CO<sub>2</sub>, contributing to the release of greenhouse gases (Cudjoe et al., 2021). These waste materials may be a good source for the preparation of PHs using specific proteases secreted by microorganisms adapted to

waste dumping yards (Shestakova et al., 2021). This comprehensive review aims to investigate the significance of utilising PHs extracted from agro-based by-products as biostimulants. This review is distinguished by its comprehensive integration of PH derived from both plant and animal sources, presenting a unified perspective on their production methodologies, biochemical properties and applications in agriculture. By linking waste valorization processes with advanced enzymatic hydrolysis techniques and agronomic outcomes, this study uniquely underscores the importance of PHs as vital elements in sustainable agriculture and the circular bioeconomy. This study explores the techniques involved in their production strategy, biochemical composition, mode of action, and implications for boosting plant growth, development, and stress tolerance. By consolidating existing research and knowledge gaps, we aim to highlight the promising potential of these sustainable and environmentally friendly compounds.

## **2. Valorization of byproducts**

PHs derived from industrial byproducts consist of agro-based protein waste from both animal and plant substances which possess considerable potential as bioactive compounds (Baglieri et al., 2014). Because PHs are made by recycling waste streams from the agriculture and food sectors, waste disposal complications are also addressed from both economic and environmental aspects (Xu and Geelen, 2018). Plant PHs are primarily generated through the enzymatic degradation of plant biomass such as alfalfa hay, legume seeds, and other plant by-products (Sestili et al., 2018a). However, they can also be produced using various methods such as partial chemical (acid or alkaline hydrolysis), enzymatic, and thermal hydrolysis (Calvo et al., 2014). Currently, various types of PHs are available in the market, which are produced by the enzymatic hydrolysis of industrial by-products that can be either animal-or plant-based proteins (Colla et al., 2017a). These sources included fish byproducts, chicken feathers, dairy byproducts, blood meal, alfalfa hay, legume seed corn mill residues, and leftovers (Fig. 1)

(Costa et al., 2023). Variability from one batch to another is caused by differences in the composition of raw materials, the methods of hydrolysis, and the extent of hydrolysis, all of which influence the consistency of bioactive peptide profiles and their effectiveness (Benítez García et al., 2025). The excessive amounts of free amino acids in certain hydrolysates can cause phytotoxicity and hinder growth due to an imbalance of amino acids and restricted nutrient absorption (Ambrosini et al., 2022). Common commercial enzymes include Alcalase (subtilisin-type endoprotease from *Bacillus licheniformis*), Neutrase (neutral protease from *Bacillus amyloliquefaciens*), papain (from papaya), bromelain (from pineapple), trypsin (from porcine/bovine pancreas), flavourzyme (from *Aspergillus oryzae*), and pepsin (from porcine stomach) (Li et al., 2025). Enzymes such as trypsin and pepsin are used to produce PH from animal sources, while plant-based hydrolysates use enzymes such as Alcalase and papain in neutral-to-acidic conditions. Enzyme selection depends on the material, desired peptide composition, and agricultural purpose for optimal yield (Inca-Torres et al., 2024). Animal-based hydrolysates contain high levels of glycine, proline, and hydroxyproline. For example, chicken feathers are interesting biostimulants that support sustainable agricultural practice. These hydrolysates sustainably repurpose poultry feather waste, reducing environmental impact while substituting synthetic fertilisers for them. Microbial biodegradation ensures safety and prevents phytotoxicity (Jagadeesan et al., 2024). Chicken feather hydrolysates act as biostimulants through keratin-based peptides and amino acids, providing organic nitrogen and signals that enhance the growth of plants. Greenhouse trials with lettuce showed increased biomass and nitrogen recovery (Sobucki et al., 2019). Hydrolysates produced from shrimp byproducts may be the best alternative to commercially available nitrogen sources. Shrimp hydrolysate has been produced using five different proteolytic enzymes: Alcalase®, trypsin, crude enzyme (*Sardinella aurita*), and serine proteases (*Aspergillus clavatus* ES1 and *Bacillus*

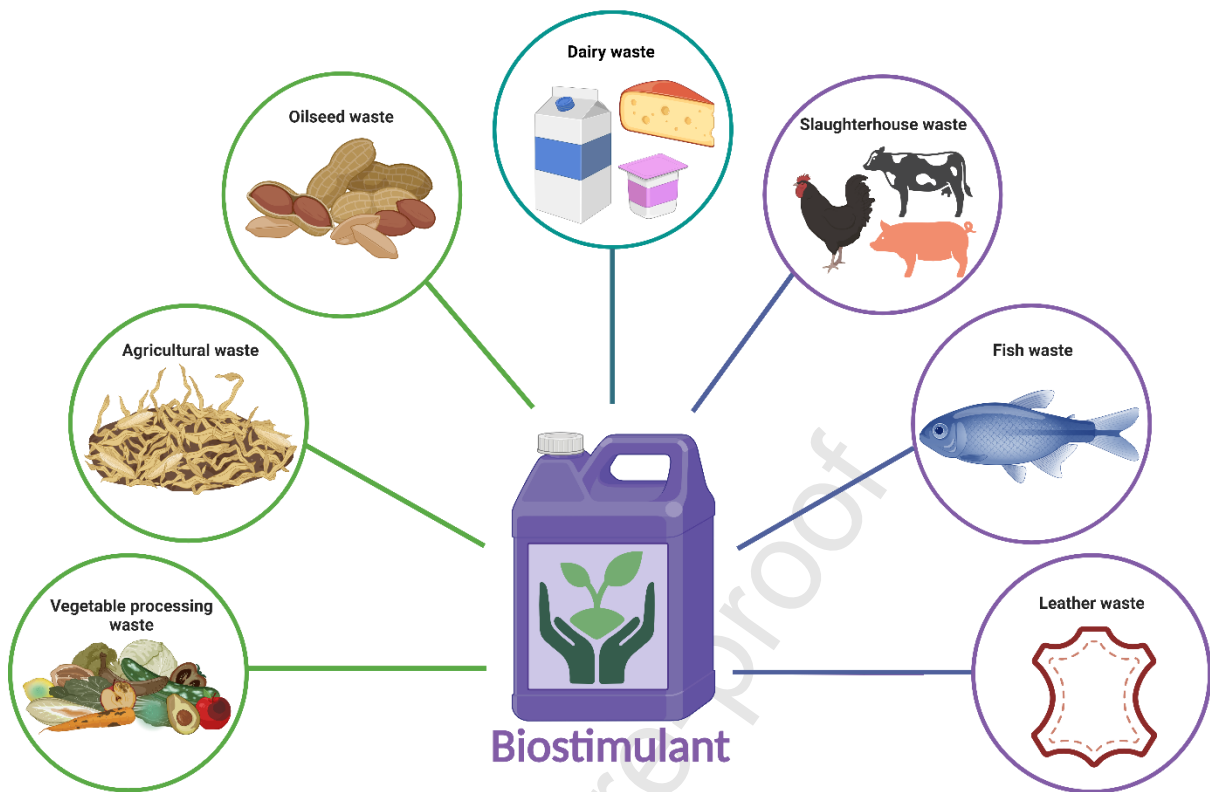
*licheniformis* NH1) (Ghorbel-Bellaaj et al., 2017). Plant-based hydrolysates are particularly rich in arginine and glutamine (Wilson et al., 2018).

Enzymatic hydrolysis is the most promising technique for sustainable PH biostimulant production, offering gentle conditions and bioactive peptide preservation while minimising environmental impacts. Enzyme-assisted methods provide scalable solutions for both plant and animal substrates. Traditional methods, such as chemical hydrolysis or high-temperature treatments, cause nutrient degradation and generate hazardous waste. Enzymatic techniques align with circular economy principles and enhance the sustainability of PH biostimulant production (Table 1).

**Table 1**

**Overview of PH production methods**

Source	Raw Materials	Conventional methods/less sustainable	Emerging methods	References
<b>Plant-derived PHs</b>	Oilseed cakes, cereal by-products, legumes, agriculture residues	Chemical hydrolysis (toxic, degradation, energy use), solvent extraction, isoelectric precipitation	Enzymatic hydrolysis produces peptides with high bioactivity, environmental impact, and valorises wastes in a safer manner than chemical routes.	(Chandran et al., 2023), (Di Serio et al., 2025), (Meena et al., 2025)
<b>Animal-derived PHs</b>	Fish waste, leather waste, blood meal, poultry feathers, dairy residues	Chemical hydrolysis (loss of essential amino acid), autoclaving, thermal treatment (non-specific cleavage)	Enzymatic hydrolysis valorizes wastes, safer than chemical routes, yields peptides with high bioactivity, reduced environmental footprint.	(Farruggia et al., 2025), (Kumar et al., 2025), (Pérez-Aguilar et al., 2024)



**Fig. 1.** Valorization of protein-rich wastes or byproducts for biostimulant production. Designed using BioRender.com.

### 3. Available PHs based biostimulants

Researchers often consider PHs, particularly those containing antioxidant peptides, as biostimulants due to their low molecular weight, ease of absorption, cost-effectiveness, high activity, and minimal side effects (Lin et al., 2017). To provide an overview of the current market for PH-based biostimulants, information on several commercial products is presented (Table 2). This table summarises the key details, including product names, manufacturing companies, target plant species, reported benefits, and relevant references. As reflected in Table 2, these biostimulants are being applied to a diverse range of crops and offer various benefits, such as improved nutrient uptake, enhanced stress tolerance, and increased yield.

**Table 2**

## PH (aminoacids and peptides) based biostimulants available in the market

Product name	Industry name	Source/Country	Plants	Benefits	References
Vegamin®	Hello Nature	Plant/Italy	Lettuce ( <i>Lactuca sativa</i> )	Increase biomass and plant resilience under reduced nitrogen conditions	(Monterisi et al., 2024a)
Trainer®	Italpollina	Plant/Italy	Basil ( <i>Ocimum basilicum</i> )	Improvement of productivity and reduced nitrate level	(Ciriello et al., 2022a)
Quik-Link®	Italpollina	Plant/Italy	Melon ( <i>Cucumis melo</i> )	Metabolite accumulation for defense mechanisms	(Lucini et al., 2018)
Hicure®	Syngenta	Animal/Switzerland	Chrysanthemum ( <i>Chrysanthemum morifolium</i> )	Increase yield and vase life of flowers; enhance abiotic stress tolerance	(Carillo et al., 2022)
Pepton 85/16®	APC Europe	Animal/Spain	Cherry tomato ( <i>Lycopersicon esculentum</i> var. <i>cerasiforme</i> )	Enhanced growth and yield under low stress condition	(Polo and Mata, 2018)
Stressal®	Bioiberica	Animal/Spain	Date plum ( <i>Diospyros lotus</i> L.)	Reduce the chloride uptake on leaves and enhance salt tolerance	(Visconti et al., 2015)
APR®	ILSA S.p.A	Animal/Italy	Maize ( <i>Zea mays</i> )	Induce molecular mechanisms by altering protein and genes related to primary and secondary metabolic pathways.	(Carletti et al., 2020)

Phytostim®	NTK	Plant/South Africa	Lettuce ( <i>Lactuca sativa</i> )	Improved yield and growth parameters	(Mpai et al., 2022)
Codasil®	Sustainable agro solutions	NA/Spain	Green beans ( <i>Phaseolus vulgaris</i> )	Water stress tolerance	(Hernández-Figueroa et al., 2024)
Terra Sorb®	Bioiberica	Plant/Spain	Mexican common bean ( <i>Phaseolus vulgaris</i> L.)	Increased the fibre level and antioxidant properties	(Kocira et al., 2020)
Isabion	Syngenta	Animal/Switzerland	Cotton ( <i>Gossypium hirsutum</i> L.)	Increased cold stress tolerance	(Ergin et al., 2024)
Actium®	Grupo Agrotecnología	NA/Spain	Sweet yellow pepper ( <i>Capsicum annuum</i> )	Carotenoid level increased and changed the fruit color	(Barrajón-Catalán et al., 2020)
Megafol®	Valagro	Plant/Italy	Sweet yellow pepper ( <i>Capsicum annuum</i> )	Increased the growth of leaves and stress tolerance	(Petrozza et al., 2014)
Radifarm®	Valagro	Plant/Italy	Sweet yellow pepper ( <i>Capsicum annuum</i> )	Increased development and growth of roots	(Parađiković et al., 2019)
Viva®	Valagro	Plant/Italy	Sweet yellow pepper ( <i>Capsicum annuum</i> )	Reduced fruit dropping and maintained fruit setting	(Bhupenchandra et al., 2022)
Benefit®	valagro	NA/Italy	Sweet yellow pepper ( <i>Capsicum annuum</i> )	Increased various metabolic reactions to maintain equal fruit size and weight	(Drobek et al., 2019)
Delfan plus	Tradecorp	Animal/Spain	Thale cress ( <i>Arabidopsis thaliana</i> )	Increased the heat stress tolerance	(Cocetta et al., 2022)

Natural Crop SL	Natural Poland	Crop	Animal/Poland	Winter wheat ( <i>Triticum aestivum L.</i> )	Increased seed quality and fatty acid content in grains	the (Cocetta et al., 2022)
Tecamin Max®	Agritecno		Plant/Spain	Okra ( <i>Abelmoschus esculentus</i> )	Increased photosynthetic efficiency, fruit quality and flower setting ratio	(Al-Shammari et al., 2019)
Tecamin flower®	Agritecno		NA/France	Tomato ( <i>Solanum lycopersicum</i> )	Enhanced fruit quality against salinity stress	the (Maach et al., 2021)
AminoHort®	Intermag		Animal/Poland	Winter wheat ( <i>Triticum aestivum L.</i> )	Increased nutrient content such as calcium, sodium, copper and molybdenum	the (Popko et al., 2018)
Agromoree	Russian Aquaculture		Animal/Russia	<i>Triticum aestivum L.</i>	Increased growth and productivity	(Mironenko et al., 2022)

#### 4. Mode of PHs' application and transport in plants

**Table 3**

##### PH and their methods of application, and doses

Waste or byproduct	Extracted substance	Application method/Plant	Dose	Plant responses	Reference
Chicken feather	Chicken feather waste hydrolysate	Root drenching/Mung bean	20 mL of PH/pot	Improved biochemical characteristics yield and growth	(Kaur et al., 2021)
Animal protein waste water	waste collagen hydrolysate	Seed germination/Chinese cabbage	0.15-0.20% of collagen hydrolysate	Improved germination rates up to 17%	(Pérez-Aguilar et al., 2023)
Chicken feather	Chicken feather protein	Root drenching/maize	CFPH - 5% w/w in 2kg soil/pot	Improved phytochemical composition,	(Jagadeesan et al., 2023)

	hydrolysate (CFPH)				yield and growth development	
Fish waste water	Biodegraded Nile perch waste water (NPWW)	Root drenching/Barley	300 mL of Biodegraded NPWW per hydroponic pots containing 20 seed	Improved photosynthetic pigment production and antioxidant activity		(Mponda and Kim, 2023)
Poultry feather	Feather hydrolysate (FH)	Root drenching/Brinjal and chilli	20% v/v FH was added at 4 days of interval up to 30 days	Improved nutritional quality, early flowering, growth and development		(Gurav et al., 2020)
Animal blood	Hydrostim biostimulant	Foliar spray/Petunia	0.2 g/L of hydrostim was used	Improved effect on biomass, gas exchange and macronutrient content		(Cristiano and De Lucia, 2021)
Whey	Pepsin hydrolysed whey protein	Foliar spray/Pea plant	2000 mg/L was given at 3 doses up to 45 days	Increased nutrient uptake and photosynthetic pigment		(Osman et al., 2021a)

PHs can be applied to plants through foliar spraying on leaves and drip irrigation root systems, as several studies have indicated that plants can absorb amino acids through their roots and leaves (Colla et al., 2015). Foliar sprays containing whey protein hydrolyzed by pepsin and papain enhanced nutrient uptake and photosynthetic pigments in pea plants, facilitating amino acid absorption through leaves (Osman et al., 2021b)(Sestili et al., 2018b). Various responses can be achieved in plants through foliar and soil treatments. Foliar sprays on leaves are typically used to attain short-term effects, whereas soil root applications are used to obtain long-term effects (Paul et al., 2019). Foliar application of PHs enhances crop productivity, stress resilience, and physiology in tomatoes (Alfosea-Simón et al., 2020), fruit trees (Tanou et al., 2017), and soybean (Teixeira et al., 2018). Surfactants aid the transport of hydrophilic amino acids and small peptides through the hydrophobic cuticle, and other factors also influence absorption. Upon entering the apoplast, plasma membrane transporters absorb amino acids and

peptides (Cannata et al., 2024). Peptides and amino acids penetrate leaves through the stomata and hydrophilic pores (i.e. polar pores), whereas in the root system, they are transported using a membrane transport system (ATP-binding cassette membrane transport (ABC)) (Boursiac et al., 2013). Amino acid transporters have been studied in various models and crop species such as *Arabidopsis*, maize (*Zea mays* L.), potato (*Solanum tuberosum*), barley (*Hordeum vulgare* L.), broad bean (*Vicia faba* L.), common bean (*Phaseolus vulgaris* L.), rice (*Oryza sativa* L.), and tomato (*Solanum lycopersicum*) (Yao et al., 2020). Uptake assays with labelled membrane vesicles from plant tissues have identified multiple amino acid and peptide transport systems. Functional analyses using heterologous expression systems, gene knockout and overexpression lines, and expression studies elucidated their roles in uptake, cellular import/export, and long-distance vascular transport (Tegeder, 2014). Amino acid transporters in plants are categorised into amino acid/auxin permease (AAAP) and Amino Acid-Polyamine-Organocation (APC) families based on sequence similarity and uptake properties. The AAAP subfamily comprises indole-3-acetic acid transporters (AUXs), amino acid transporter-like proteins, amino acid permeases (AAPs),  $\gamma$ -aminobutyric acid transporters (GATs), lysine and histidine transporters (LHTs), proline transporters (ProTs), aromatic amino acid transporters, and neutral amino acid transporters. The APC family comprises polyamine H<sup>+</sup>symporters (PHSs), amino acid/choline transporters, and cationic amino acid transporters (CATs) (Yao et al., 2020)(Tegeder and Masclaux-Daubresse, 2018). Another class of transporters known as 'usually multiple acids move in and out transporters' (UMAMIT) was discovered in *Arabidopsis thaliana* (Ranocha et al., 2013).

In plants, three gene families are specialized in transporting peptides of specific lengths. The peptide transporter/nitrate transporter 1 (PTR/NRT1) family handles di- and tri-peptides. The OPT oligopeptide transporter (OPT) family transports peptides with four to five amino acids. Larger peptides are transported by ATP-binding cassette (ABC) transporters (Yuan et al., 2024).

Plant cell membranes contain leucine-rich repeat receptor kinases (LRR-RKs) that detect protein and peptide ligands. These receptors are crucial for plant immune defence and, in roots, they help regulate the formation of diffusion barriers in the Casparian strip located in the centre of the root endodermis of vascular plants (Furumizu and Aalen, 2023). PHs in nutrient solutions are dose-dependent and have direct contact with the root area which could increase nutrient uptake and assimilation *via* the root modification system, such as structural and functional modifications to adapt to the adverse environmental conditions in “Genovese” basil for example (Ciriello et al., 2022b). In addition to surfactants and transporter families, factors such as soil pH, microbial competition, temperature, moisture, and the composition of soil organic nitrogen play crucial roles in determining the availability and absorption of amino acids in plants. When soil becomes more acidic, it alters microbial communities and enzyme activity, affecting amino acid mineralization and plant-microorganism competition for amino acids (Pan et al., 2022). Table 3 summarises the key findings of various studies on PHs, detailing their sources, application methods, dosages, and benefits. As evident from the compiled data, PHs derived from both animal and plant sources have demonstrated remarkable efficacy across a range of crops. Considering the diverse effects of PHs as biostimulants, our review focuses primarily on elucidating the underlying mechanisms of action that potentially explain the broad spectrum of PH-induced plant responses. This approach aims to identify the common physiological and molecular pathways that may be universally activated or modulated by PHs across various plant species and environmental conditions.

## **5. Mechanisms of action of PH under abiotic stresses**

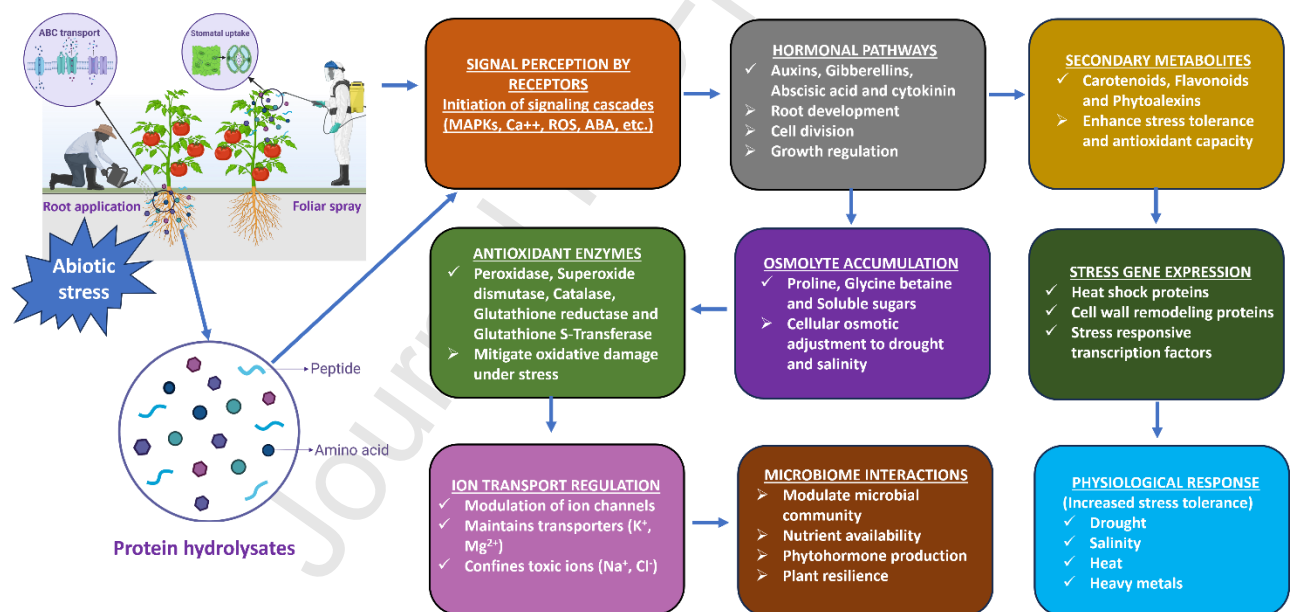
Crop production and yield are significantly affected by non-living environmental factors (abiotic factors). These include extreme temperatures (both high and low), salinity, drought, environmental contamination, presence of metalloids and heavy metals, insufficient nutrients, excessive light exposure, and ultraviolet (UV) radiation (Wahab et al., 2022). PHs enhance

tolerance to abiotic stress when applied to plant roots or leaves (Lucini et al., 2015). PH plays a crucial role in plant growth and bolsters resistance to abiotic stress through a sophisticated array of molecular signalling pathways. These pathways include hormone-like actions, antioxidant defences, osmotic balance, and regulation of gene expression. PHs are composed of bioactive peptides and amino acids that act as signalling molecules, triggering hormone pathways, such as auxin and gibberellin. This activation enhances root growth, nutrient absorption, and photosynthetic performance (Colla et al., 2017a). PHs enhance antioxidant enzyme activity for cellular defense during stress, while influencing osmolyte accumulation and ion transporters crucial for stability under drought and salinity (Monterisi et al., 2024b). Transcriptomic and proteomic studies have shown that PHs trigger the expression of genes involved in cell wall restructuring, stress signalling, and heat shock proteins, allowing plants to better withstand temperature fluctuations and oxidative stress (Carillo, 2025). PHs' interactions with the plant microbiome bolster stress resistance by altering microbial communities that enhance nutrient and phytohormone production. These interconnected pathways create a signalling network, establishing PHs as biostimulants that enhance plant health under challenging conditions (Fig. 2)(Malécange et al., 2023). Tomato plants treated with Plant-derived Trainer® PHs exhibited improved equilibrium between photosynthetic processes and water retention during drought periods. This equilibrium enables plants to maintain their functionality and resilience under stressful conditions. Consequently, when water becomes accessible, plants are in a better position to recover quickly, resulting in less severe damage from drought-induced stress (Paul et al., 2019). In addition, this commercial PH can increase the nitrogen content by enhancing the expression of genes in tomatoes that are involved in nitrogen assimilation, particularly under low nitrogen conditions, enhancing the gene expression responsible for amino acid transport and ferredoxin-GOGAT (glutamate synthases), as well as glutamine synthetase in root tissues. Conversely, the expression of genes

associated with ammonium and nitrate transporters, along with nitrate reductase, is suppressed, particularly in leaf tissues (Sestili et al., 2018a). PHs can induce greater photosynthetic efficiency by increasing the total chlorophyll index (Van Oosten et al., 2017). It can also modulate leaf gas exchange and water-use efficiency. During drought, osmotic adjustment is achieved by elevating the concentrations of proline and total soluble sugars, thus ensuring membrane stability and enabling proteins to maintain their function (Zulfiqar et al., 2020). In the context of salt stress, the production and accumulation of compounds such as proline and glycine betaine, along with other proteins such as Late Embryogenesis Abundant (LEA) proteins, HSPs, dehydrins, receptor-like kinases, and aquaporins, in response to high-salinity conditions, demonstrate how PHs can generate various genetic characteristics that enable adaptation to salt stress. Recent research has focused on improving salt tolerance in naturally salt-sensitive *Diospyros lotus* (*D. lotus*) rootstocks. This approach combines two strategies: (1) exploiting the genetic variability in salt tolerance found among different *Diospyros* species through breeding programs and (2) applying PHs treatments as biostimulants. By crossbreeding *D. lotus* with more salt-tolerant relatives such as *D. virginiana* and selecting for salt-tolerant traits, researchers have aimed to develop new rootstock varieties with enhanced salt tolerance. The application of commercial calcium PHs may further boost the stress resilience of improved rootstocks (Gil-Muñoz et al., 2020)(Visconti et al., 2015). This adaptation, either through root or leaf treatment, could lead to the accumulation of glucosinolates, terpenes, sterols, and osmolytes in salt-stressed lettuce plants (Lucini et al., 2015). This process can reduce oxidative stress, improve nitrogen uptake efficiency, and enhance photosynthetic properties. In another study on lettuce, PHs increased the efficiency of photosynthesis under saline conditions to maintain Mg and K<sup>+</sup> levels in the cytosol while simultaneously confining the high concentration of toxin ions (sodium, chloride, and sulfate) inside the vacuoles (Rouphael et al., 2017). This evidence suggests that the use of PH-based biostimulants helps maintain certain

types of active metabolism under salt stress. The metabolic fingerprint of salt-stressed plants involves the aggregation of stress-related molecules, mostly jasmonates, glucosinolates, and terpenoid phytoalexins which can be alleviated by PH application (Rouphael et al., 2022). Transcriptomic and proteomic studies on commercial PHs biostimulants (ILSA S.p.A, Arzignano, Vicenza, Italy) showed a dose-dependent stimulant effect against abiotic stresses, such as salt and drought, through the expression of differentially expressed genes involved in root growth (polygalacturonases, root cap genes, and fasciclin-like arabinogalactan proteins) and differentially abundant proteins involved in cell wall growth (expansins, villins, cell division control proteins, and tubulin) (Carletti et al., 2020). Transcriptomic data also revealed that the gene expression of different small HSPs plays an important role in thermotolerance under extreme heat conditions in maize crops upon foliar application of PHs produced from animal residues (SICIT, Italy) (Santi et al., 2017). Another PHs (Terra-Sorb Foliar®) increased carotenoid levels, chlorophyll content, and photosynthetic efficiency in ryegrass (*Lolium perenne* L.) under heat stress conditions (Vaseva et al., 2022). Another study demonstrated that an amino acid-based biostimulant affected the uptake of nutrients by cannabis plants and influenced the levels of cannabinoids and terpenes in flowers. This suggests that PHs biostimulants have a positive effect on the production of secondary metabolites (Malík et al., 2022). Additionally, amino acid-based biostimulants lower the energy required for the production of endogenous amino acids and promote plant growth whereas peptide-based biostimulants act as signal-transduction molecules (Zhang et al., 2023). The alfalfa PHs increased the intensity of red colour of apple “Jonathan” and also increased its shelf life periods during storage (Soppelsa et al., 2018). Specifically, alfalfa-derived PHs have been found to increase the levels of ascorbic acid (vitamin C), flavonoids, and carotenoids when incorporated into plant leaves, thereby improving the gustative quality parameters in fruits (Ertani et al., 2017).

Several studies have indicated that biostimulants enhance plant resilience, particularly by increasing the antioxidant activity inside the plant during adverse environmental conditions (González-Morales et al., 2021). Amino acids decrease the toxicity of plants caused by hazardous metals (heavy metals) by acting as chelators of these metals (Syta et al., 2013). Animal-derived gelatin, which is rich in amino acids and peptides, has been shown to enhance the dry weight of shoots and boost nitrogen assimilation by roots in a range of plants, including arugula, broccoli, corn, cucumber, pepper, and tomato (Wilson et al., 2018). Plant-derived PH-based biostimulants exhibit hormone-like activity by promoting crosstalk signalling between phytohormones (auxins, gibberellins, and cytokinins) during root development in tomato plants (Ceccarelli et al., 2021b).



**Fig. 2.** Mechanisms of action of PH-based biostimulants and plant responses. The figure shows the expression of signaling pathway involving Mitogen-Activated Protein Kinases (MAPKs), Calcium ions (Ca<sup>++</sup>), Reactive Oxygen Species (ROS) and Abscisic Acid (ABA) in plant stress response. Functional roles in ion homeostasis: maintaining nutrient transporters such as potassium (K<sup>+</sup>) and magnesium (Mg<sup>2+</sup>), while confining toxic ions like sodium (Na<sup>+</sup>) and chloride (Cl<sup>-</sup>). Designed using BioRender.com

## 6. Microbiome interactions

Plants are colonised by an abundant and diverse assortment of microbial taxa that help them acquire nutrients and water and withstand biotic and abiotic stress. The substrates provided by PHs, such as amino acids, could provide an ideal food source for these plant-associated microbes (Wise et al., 2024). A diverse and plentiful array of microbial species inhabit plants and offer numerous benefits to their hosts. These microorganisms aid in the uptake of water and nutrients, promotes the stress tolerance against both biotic and abiotic conditions and also protects from pathogenic attack (Lau et al., 2022). The components found in PHs, particularly amino acids, may serve as an excellent nutritional source for these plant-associated microbes. Indeed, the literature suggests that PHs can alter the microbial community and its function, potentially contributing to the advantageous outcomes observed following the application of these substances (Mattarozzi et al., 2020)(Ganugi et al., 2022). Recently, researchers have discovered that microorganisms coping with biological and environmental stressors are also present in the phyllosphere, which covers the surfaces of plant leaves. The compounds found in PHs could serve as nitrogen and carbon sources for microbes inhabiting both the rhizosphere and phyllosphere of plants (Colla et al., 2017b)(Zhou et al., 2024). The predominant phyla in the rhizosphere and phyllosphere of common bean (*Phaseolus vulgaris*), soybean (*Glycine max*), tomato (*Solanum lycopersicum*), cucumber (*Cucumis sativus*), and maize (*Zea mays*) include *Proteobacteria* (*Rhizobium*, *Mesorhizobium*, *Azospirillum*, *Bradyrhizobium*, *Pseudomonas*, and *Sphingomonas*), *Bacteroidetes* (*Flavobacterium*, *Arenibacter*, and *Algoriphagus*), and *Acidobacteria* (*Luteolibacter*, *Candidatus Rhizosphaeria*). Fungal communities are also significant, particularly in plants such as barley (*Hordeum vulgare*) and wheat (*Triticum aestivum*), with the major phyla including *Ascomycota* (*Fusarium*, *Penicillium*, *Trichoderma*, and *Alternaria*) and *Basidiomycota* (*Agaricales*, *Auriculariales*, and *Sporidiobolales*) (Viebahn et al., 2005)(Qiao et al., 2019). Previous studies have indicated that

casein and soybean hydrolysates can activate defence mechanisms in grapevines, reducing the incidence of downy mildew induced by *Plasmopara viticola* (Boselli et al., 2019). A root hair-promoting peptide was produced from soybean hydrolysate using alkaline protease produced from *Bacillus circulans* HA12. The foliar application of the PH-based biostimulants Trainer<sup>®</sup> and Auxym<sup>®</sup> revealed alterations in the phyllosphere microbial community (Colla et al., 2017b). Furthermore, these results offer intriguing support for the notion that such products could be tailored to foster beneficial interactions between plants and microbes, thereby improving plant productivity.

## 7. Future perspectives

The development of tailored PH-based biostimulants is essential for maximising crop efficacy across growth phases and environmental conditions. Further investigations are needed to understand PHs underlying the enhanced nutrient uptake in plants by PHs, which may potentially reduce the dependence on chemical fertilisers. A sustainable approach requires the exploration of novel protein sources from agricultural by-products to create viable PH biostimulants. Examining the synergistic effects between PHs and other biostimulants could enhance plant growth promotion. Innovative application techniques are crucial for improving the efficiency of biostimulant delivery. Future research should focus on adapting biostimulants to enhance crop resilience under changing climatic conditions. Further investigation is needed to understand the molecular mechanisms by which PH stimulates plant growth and stress tolerance. Advanced metabolomic and proteomic technologies will provide a better understanding of PHs' influence of PHs on plant metabolism. The effects of PHs on plant microbiomes require further examination to determine their contributions to plant growth. Further research is needed to determine the effective amino acid and peptide combinations in PH formulations for specific crops. Regulatory authorities in the European Union, the United States, and other regions are endeavouring to establish precise definitions for biostimulants,

differentiating them from fertilisers and pesticides. This effort aims to ensure product safety while fostering innovations. The current regulatory ambiguity underscores the necessity for research and policy development to facilitate the sustainable integration of plant health biostimulants into agricultural systems.

## **7. Conclusion**

The requirement to shift agriculture towards higher self-sufficiency in terms of chemical inputs has prompted the entire agricultural industry to look for sustainable and efficient alternatives. PHs differ from seaweed extracts, microbial inoculants, and humic substances. PHs contain peptides and amino acids that enhance nutrient efficiency. Seaweed biostimulants provide polysaccharides for growth, whereas microbial biostimulants improve nutrient content. Humic substances improve soil structure, whereas PHs enhance microbial activity, stress tolerance, and plant growth. The use of PH biostimulant products is a potential solution for long-term agriculture. The current literature mainly focuses on the significance of PH-based biostimulants, such as their mode of application, to evaluate their true potentials. The outline reported in this review emphasises the necessity of interdisciplinary research on biostimulants. These substances substantially impact the production of plant metabolites (primary and secondary metabolites), which are significantly induced by numerous bioactive components with synergistic effects. Before commercialising or using a novel biostimulant substance, it is essential to meet the guidelines and regulations for biostimulant use to prevent adverse effects and safeguard the environment and consumers. It is important for farmers to evaluate the financial viability of integrating biostimulants into their farming practices, considering both the expenses and advantages that may arise. Further research is necessary to provide a brief and comprehensive understanding of these PH-based biostimulants and their potential applications in economically important crops and leafy vegetables, including lettuce. This review offers a comprehensive overview of PH sourced from various biological origins and

emphasises their sustainable utilisation, thereby laying the groundwork for future research and application of biostimulants to enhance robust and efficient agricultural systems.

### **CRedit authorship contribution statement**

Padmapriya Dhanasekaran: Writing – original draft, Methodology, Investigation, Tabulation.

Jane Roche: Writing – review & editing, Conceptualization, Validation, Formal analysis.

Shanthi Chittibabu, Aurélia Stevens, Said Mouzeyar, and Cédric Delattre: review & editing.

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The authors declare that they have no conflicts of interest.

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This study did not generate or analyse novel data. The concept of data sharing is not applicable to this article.

### **References**

- Alfosea-Simón, M., Zavala-Gonzalez, E.A., Camara-Zapata, J.M., Martínez-Nicolás, J.J., Simón, I., Simón-Grao, S., García-Sánchez, F., 2020. Effect of foliar application of amino acids on the salinity tolerance of tomato plants cultivated under hydroponic system. *Sci Hort* 272. <https://doi.org/10.1016/j.scienta.2020.109509>
- Al-Shammari, A.M.A., Abood, M.A., Hamdi, G.J., 2019. Foliar application of Tecamin max® to alleviate water deficit on vegetative growth and yield of okra. *International Journal of Vegetable Science* 25, 278–284. <https://doi.org/10.1080/19315260.2018.1509922>
- Ambrosini, S., Prinsi, B., Zamboni, A., Espen, L., Zanzoni, S., Santi, C., Varanini, Z., Pandolfini, T., 2022. Chemical Characterization of a Collagen-Derived Protein Hydrolysate and Biostimulant Activity Assessment of Its Peptidic Components. *J Agric Food Chem* 70, 11201–11211. <https://doi.org/10.1021/acs.jafc.2c04379>
- Antón-Herrero, R., García-Delgado, C., Antón-Herrero, G., Mayans, B., Delgado-Moreno, L., Eymar, E., 2023. Design of a hydroponic test to evaluate the biostimulant potential of new organic and organomineral products. *Sci Hort* 310. <https://doi.org/10.1016/j.scienta.2022.111753>
- Baglieri, A., Cadili, V., Mozzetti Monterumici, C., Gennari, M., Tabasso, S., Montoneri, E., Nardi, S., Negre, M., 2014. Fertilization of bean plants with tomato plants hydrolysates. Effect on biomass production, chlorophyll content and N assimilation. *Sci Hort* 176, 194–199. <https://doi.org/10.1016/j.scienta.2014.07.002>
- Barrajón-Catalán, E., Álvarez-Martínez, F.J., Borrás, F., Pérez, D., Herrero, N., Ruiz, J.J., Micol, V., 2020. Metabolomic analysis of the effects of a commercial complex biostimulant on pepper crops. *Food Chem* 310. <https://doi.org/10.1016/j.foodchem.2019.125818>

Benítez García, I., Álvarez Armendariz, B.M., Osuna Ruiz, I., Esparza Araiza, M.J., Salazar Leyva, J.A., Rios Herrera, G.D., Martínez Montaña, E., 2025. Acid Silage from Nile Tilapia (*Oreochromis niloticus*) Byproducts as a Potential Biostimulant of Bread Wheat (*Triticum aestivum*) Sprouts. Waste Biomass Valorization. <https://doi.org/10.1007/s12649-025-03148-9>

Bhupenchandra, I., Chongtham, S.K., Devi, E.L., Ramesh, R., Choudhary, A.K., Salam, M.D., Sahoo, M.R., Bhutia, T.L., Devi, S.H., Thounaojam, A.S., Behera, C., Harish, M.N., Kumar, A., Dasgupta, M., Devi, Y.P., Singh, D., Bhagowati, S., Devi, C.P., Singh, H.R., Khaba, C.I., 2022. Role of biostimulants in mitigating the effects of climate change on crop performance. Front Plant Sci. <https://doi.org/10.3389/fpls.2022.967665>

Boselli, M., Bahouaoui, M.A., Lachhab, N., Sanzani, S.M., Ferrara, G., Ippolito, A., 2019. Protein hydrolysates effects on grapevine (*Vitis vinifera* L., cv. Corvina) performance and water stress tolerance. Sci Horti 258. <https://doi.org/10.1016/j.scienta.2019.108784>

Boursiac, Y., Lérans, S., Corratgé-Faillie, C., Gojon, A., Krouk, G., Lacombe, B., 2013. ABA transport and transporters. Trends Plant Sci. <https://doi.org/10.1016/j.tplants.2013.01.007>

Buffagni, V., Ceccarelli, A.V., Pii, Y., Miras-Moreno, B., Rouphael, Y., Cardarelli, M., Colla, G., Lucini, L., 2021. The modulation of auxin-responsive genes, phytohormone profile, and metabolomic signature in leaves of tomato cuttings is specifically modulated by different protein hydrolysates. Agronomy 11. <https://doi.org/10.3390/agronomy11081524>

Calvo, P., Nelson, L., Kloepper, J.W., 2014. Agricultural uses of plant biostimulants. Plant Soil. <https://doi.org/10.1007/s11104-014-2131-8>

- Cannata, C., Basile, F., La Bella, E., Arciello, S., Abreu, A.C., Fernández, I., Leonardi, C., Mauro, R.P., 2024. Exhausted plant cell culture media as potential biostimulants to enhance plant growth and nitrogen use efficiency in tomatoes under optimal and reduced nitrogen supply. *Journal of Plant Nutrition and Soil Science*.  
<https://doi.org/10.1002/jpln.202300464>
- Carillo, P., 2025. Can biostimulants enhance plant resilience to heat and water stress in the Mediterranean hotspot? *Plant Stress*. <https://doi.org/10.1016/j.stress.2025.100802>
- Carillo, P., Pannico, A., Cirillo, C., Ciriello, M., Colla, G., Cardarelli, M., De Pascale, S., Rouphael, Y., 2022. Protein Hydrolysates from Animal or Vegetal Sources Affect Morpho-Physiological Traits, Ornamental Quality, Mineral Composition, and Shelf-Life of Chrysanthemum in a Distinctive Manner. *Plants* 11.  
<https://doi.org/10.3390/plants11172321>
- Carletti, P., Arrigoni, G., Ebinezer, L.B., Franchin, C., Trentin, A.R., Trevisan, S., Agrawal, G.K., Rakwal, R., Quaggiotti, S., Masi, A., 2020. Quantitative proteomics of maize roots treated with a protein hydrolysate: A comparative study with transcriptomics highlights the molecular mechanisms responsive to biostimulants. *J Agric Food Chem* 68, 7541–7553. <https://doi.org/10.1021/acs.jafc.0c01593>
- Ceccarelli, A.V., Miras-Moreno, B., Buffagni, V., Senizza, B., Pii, Y., Cardarelli, M., Rouphael, Y., Colla, G., Lucini, L., 2021a. Foliar application of different vegetal-derived protein hydrolysates distinctively modulates tomato root development and metabolism. *Plants* 10, 1–15. <https://doi.org/10.3390/plants10020326>
- Ceccarelli, A.V., Miras-Moreno, B., Buffagni, V., Senizza, B., Pii, Y., Cardarelli, M., Rouphael, Y., Colla, G., Lucini, L., 2021b. Foliar application of different vegetal-derived

protein hydrolysates distinctively modulates tomato root development and metabolism.

Plants 10, 1–15. <https://doi.org/10.3390/plants10020326>

Chandran, A.S., Suri, S., Choudhary, P., 2023. Sustainable plant protein: an up-to-date overview of sources, extraction techniques and utilization. Sustainable Food Technology. <https://doi.org/10.1039/d3fb00003f>

Ciriello, M., Formisano, L., El-Nakhel, C., Corrado, G., Rouphael, Y., 2022a. Biostimulatory Action of a Plant-Derived Protein Hydrolysate on Morphological Traits, Photosynthetic Parameters, and Mineral Composition of Two Basil Cultivars Grown Hydroponically under Variable Electrical Conductivity. Horticulturae 8. <https://doi.org/10.3390/horticulturae8050409>

Ciriello, M., Fusco, G.M., Woodrow, P., Carillo, P., Rouphael, Y., 2024. Unravelling the nexus of plant response to non-microbial biostimulants under stress conditions. Plant Stress. <https://doi.org/10.1016/j.stress.2024.100421>

Ciriello, M., Kyriacou, M.C., De Pascale, S., Rouphael, Y., 2022b. An appraisal of critical factors configuring the composition of basil in minerals, bioactive secondary metabolites, micronutrients and volatile aromatic compounds. Journal of Food Composition and Analysis. <https://doi.org/10.1016/j.jfca.2022.104582>

Cocetta, G., Landoni, M., Pilu, R., Repiso, C., Nolasco, J., Alajarin, M., Ugena, L., Levy, C.C.B., Scatolino, G., Villa, D., Ferrante, A., 2022. Priming Treatments with Biostimulants to Cope the Short-Term Heat Stress Response: A Transcriptomic Profile Evaluation. Plants 11. <https://doi.org/10.3390/plants11091130>

Colla, G., Hoagland, L., Ruzzi, M., Cardarelli, M., Bonini, P., Canaguier, R., Rouphael, Y., 2017a. Biostimulant action of protein hydrolysates: Unraveling their effects on plant physiology and microbiome. Front Plant Sci. <https://doi.org/10.3389/fpls.2017.02202>

- Colla, G., Hoagland, L., Ruzzi, M., Cardarelli, M., Bonini, P., Canaguier, R., Rouphael, Y., 2017b. Biostimulant action of protein hydrolysates: Unraveling their effects on plant physiology and microbiome. *Front Plant Sci.* <https://doi.org/10.3389/fpls.2017.02202>
- Colla, G., Nardi, S., Cardarelli, M., Ertani, A., Lucini, L., Canaguier, R., Rouphael, Y., 2015. Protein hydrolysates as biostimulants in horticulture. *Sci Hortic.* <https://doi.org/10.1016/j.scienta.2015.08.037>
- Costa, O.Y.A., Pijl, A., Houbraken, J., van Lith, W., Kuramae, E.E., 2023. Soil substrate source drives the microbes involved in the degradation of gelatin used as a biostimulant. *Applied Soil Ecology* 189, 104906. <https://doi.org/10.1016/j.apsoil.2023.104906>
- Cristiano, G., De Lucia, B., 2021. Petunia Performance Under Application of Animal-Based Protein Hydrolysates: Effects on Visual Quality, Biomass, Nutrient Content, Root Morphology, and Gas Exchange. *Front Plant Sci* 12. <https://doi.org/10.3389/fpls.2021.640608>
- Cudjoe, D., Han, M.S., Chen, W., 2021. Power generation from municipal solid waste landfilled in the Beijing-Tianjin-Hebei region. *Energy* 217. <https://doi.org/10.1016/j.energy.2020.119393>
- Di Serio, A., Aquino, G., Prete, F. Del, Sansone, F., Salviati, E., Basilicata, M.G., Manfra, M., Campiglia, P., Ronga, D., Pepe, G., 2025. Protein hydrolysates derived from residual after polysaccharide extraction of *Chlorella vulgaris* biomass improves yield and quality of baby leaf lettuce. *Sci Rep* 15. <https://doi.org/10.1038/s41598-025-15748-5>
- Drobek, M., Fraç, M., Cybulska, J., 2019. Plant biostimulants: Importance of the quality and yield of horticultural crops and the improvement of plant tolerance to abiotic stress-a review. *Agronomy* 9. <https://doi.org/10.3390/agronomy9060335>

- Ebinezer, L.B., Franchin, C., Trentin, A.R., Carletti, P., Trevisan, S., Agrawal, G.K., Rakwal, R., Quaggiotti, S., Arrigoni, G., Masi, A., 2020. Quantitative Proteomics of Maize Roots Treated with a Protein Hydrolysate: A Comparative Study with Transcriptomics Highlights the Molecular Mechanisms Responsive to Biostimulants.  
<https://doi.org/10.1021/acs.jafc.0c01593>
- Ergin, N., Kulan, E.G., Harmanci, P., Kaya, M.D., 2024. The ability of biostimulants and copper-containing fungicide to protect cotton against chilling stress. *Journal of Cotton Research* 7. <https://doi.org/10.1186/s42397-024-00183-0>
- Ertani, A., Schiavon, M., Nardi, S., 2017. Transcriptome-wide identification of differentially expressed genes in *Solanum lycopersicon* L. In response to an Alfalfa-protein hydrolysate using microarrays. *Front Plant Sci* 8.  
<https://doi.org/10.3389/fpls.2017.01159>
- Farruggia, D., Licata, M., Di Miceli, G., Urso, G., Salamone, F., Novak, J., 2025. Animal and plant-derived protein hydrolysates positively affect yield traits but produce contrasting response on chemicals of organic rosemary (*Salvia rosmarinus* Spenn.) grown under rainfed conditions. *Italian Journal of Agronomy* 20.  
<https://doi.org/10.1016/j.ijagro.2025.100048>
- Flaibam, B., da Silva, M.F., de Mélo, A.H.F., Carvalho, P.H., Galland, F., Pacheco, M.T.B., Goldbeck, R., 2024. Non-animal protein hydrolysates from agro-industrial wastes: A prospect of alternative inputs for cultured meat. *Food Chem* 443.  
<https://doi.org/10.1016/j.foodchem.2024.138515>
- Furumizu, C., Aalen, R.B., 2023. Peptide signaling through leucine-rich repeat receptor kinases: insight into land plant evolution. *New Phytologist* 238, 977–982.  
<https://doi.org/10.1111/nph.18827>

- Ganugi, P., Fiorini, A., Ardenti, F., Caffi, T., Bonini, P., Taskin, E., Puglisi, E., Tabaglio, V., Trevisan, M., Lucini, L., 2022. Nitrogen use efficiency, rhizosphere bacterial community, and root metabolome reprogramming due to maize seed treatment with microbial biostimulants. *Physiol Plant* 174. <https://doi.org/10.1111/ppl.13679>
- Ghorbel-Bellaaj, O., Jellouli, K., Maalej, H., 2017. Shrimp processing by-products protein hydrolysates: Evaluation of antioxidant activity and application in biomass and proteases production. *Biocatal Biotransformation* 35, 287–297. <https://doi.org/10.1080/10242422.2017.1334766>
- Gil-Muñoz, F., Pérez-Pérez, J.G., Quiñones, A., Primo-Capella, A., Cebolla, J., Ángeles Forner-Giner, M., Badenes, M.L., del Mar Naval, M., 2020. A cross population between *D. Kaki* and *D. Virginiana* shows high variability for saline tolerance and improved salt stress tolerance. *PLoS One* 15. <https://doi.org/10.1371/journal.pone.0229023>
- González-Morales, S., Solís-Gaona, S., Valdés-Caballero, M.V., Juárez-Maldonado, A., Loredó-Treviño, A., Benavides-Mendoza, A., 2021. Transcriptomics of Biostimulation of Plants Under Abiotic Stress. *Front Genet.* <https://doi.org/10.3389/fgene.2021.583888>
- Gupta, S., Doležal, K., Kulkarni, M.G., Balázs, E., Van Staden, J., 2022. Role of non-microbial biostimulants in regulation of seed germination and seedling establishment. *Plant Growth Regul.* <https://doi.org/10.1007/s10725-021-00794-6>
- Gurav, R., Nalavade, V., Aware, C., Vyavahare, G., Bhatia, S.K., Yang, Y.H., Bapat, V., Jadhav, J., 2020. Microbial degradation of poultry feather biomass in a constructed bioreactor and application of hydrolysate as bioenhancer to vegetable crops. *Environmental Science and Pollution Research* 27, 2027–2035. <https://doi.org/10.1007/s11356-019-06536-6>

- Hernández-Figueroa, K.I., Sánchez, E., Ramírez-Estrada, C.A., Anchondo-Páez, J.C., Ojeda-Barrios, D.L., Pérez-Álvarez, S., 2024. Efficacy and Differential Physiological–Biochemical Response of Biostimulants in Green Beans Subjected to Moderate and Severe Water Stress. *Crops* 4, 27–42. <https://doi.org/10.3390/crops4010003>
- Inca-Torres, R., Aguilera-Velázquez, J.R., Urbina-Salazar, A. del R., Carbonero-Aguilar, P., Moreno Navarro, I., Bautista Palomas, J., 2024. Enzymatic Preparation of Mushroom By-product Protein Hydrolysates (Mb-PPHs). *Waste Biomass Valorization* 15, 1509–1518. <https://doi.org/10.1007/s12649-023-02157-w>
- Jagadeesan, Y., Meenakshisundaram, S., Pichaimuthu, S., Balaiah, A., 2024. A scientific version of understanding “Why did the chickens cross the road”? – A guided journey through *Bacillus* spp. towards sustainable agriculture, circular economy and biofortification. *Environ Res.* <https://doi.org/10.1016/j.envres.2023.117907>
- Jagadeesan, Y., Meenakshisundaram, S., Raja, K., Balaiah, A., 2023. Sustainable and efficient-recycling approach of chicken feather waste into liquid protein hydrolysate with biostimulant efficacy on plant, soil fertility and soil microbial consortium: A perspective to promote the circular economy. *Process Safety and Environmental Protection* 170, 573–583. <https://doi.org/10.1016/j.psep.2022.12.029>
- Kaur, M., Bhari, R., Singh, R.S., 2021. Chicken feather waste-derived protein hydrolysate as a potential biostimulant for cultivation of mung beans. *Biologia (Bratisl)* 76, 1807–1815. <https://doi.org/10.1007/s11756-021-00724-x>
- Kocira, S., Szparaga, A., Hara, P., Treder, K., Findura, P., Bartoš, P., Filip, M., 2020. Biochemical and economical effect of application biostimulants containing seaweed extracts and amino acids as an element of agroecological management of bean cultivation. *Sci Rep* 10. <https://doi.org/10.1038/s41598-020-74959-0>

- Kumar, S., Karthik, C., Govindan, P., Sharma, R.K., Padikasan, I.A., 2025. Evaluating the Biostimulating Potential of Whey Protein Hydrolysate (WPH) in *Solanum lycopersicum*: A Comprehensive Investigation. Waste Biomass Valorization. <https://doi.org/10.1007/s12649-025-02912-1>
- Lau, S.E., Teo, W.F.A., Teoh, E.Y., Tan, B.C., 2022. Microbiome engineering and plant biostimulants for sustainable crop improvement and mitigation of biotic and abiotic stresses. Discover Food. <https://doi.org/10.1007/s44187-022-00009-5>
- Li, Q., Chang, B., Huang, G., Wang, D., Gao, Y., Fan, Z., Sun, H., Sui, X., 2025. Differential Enzymatic Hydrolysis: A Study on Its Impact on Soy Protein Structure, Function, and Soy Milk Powder Properties. Foods 14. <https://doi.org/10.3390/foods14050906>
- Lin, H.C., Alashi, A.M., Aluko, R.E., Pan, B.S., Chang, Y.W., 2017. Antihypertensive properties of tilapia (*Oreochromis spp.*) frame and skin enzymatic protein hydrolysates. Food Nutr Res 61. <https://doi.org/10.1080/16546628.2017.1391666>
- Ling, Z., Thakur, N., El-Dalatony, M.M., Salama, E.S., Li, X., 2022. Protein biomethanation: insight into the microbial nexus. Trends Microbiol. <https://doi.org/10.1016/j.tim.2021.06.004>
- Lucini, L., Roupael, Y., Cardarelli, M., Bonini, P., Baffi, C., Colla, G., 2018. A vegetal biopolymer-based biostimulant promoted root growth in melon while triggering brassinosteroids and stress-related compounds. Front Plant Sci 9. <https://doi.org/10.3389/fpls.2018.00472>
- Lucini, L., Roupael, Y., Cardarelli, M., Canaguier, R., Kumar, P., Colla, G., 2015. The effect of a plant-derived biostimulant on metabolic profiling and crop performance of lettuce grown under saline conditions. Sci Hortic 182, 124–133. <https://doi.org/10.1016/j.scienta.2014.11.022>

- Maach, M., Boudouasar, K., Akodad, M., Skalli, A., Moumen, A., Baghour, M., 2021. Application of biostimulants improves yield and fruit quality in tomato. *International Journal of Vegetable Science* 27, 288–293. <https://doi.org/10.1080/19315260.2020.1780536>
- MacLaren, C., Mead, A., van Balen, D., Claessens, L., Etana, A., de Haan, J., Haagsma, W., Jäck, O., Keller, T., Labuschagne, J., Myrbeck, Å., Nepalova, M., Nziguheba, G., Six, J., Strauss, J., Swanepoel, P.A., Thierfelder, C., Topp, C., Tshuma, F., Verstegen, H., Walker, R., Watson, C., Wesselink, M., Storkey, J., 2022. Long-term evidence for ecological intensification as a pathway to sustainable agriculture. *Nat Sustain* 5, 770–779. <https://doi.org/10.1038/s41893-022-00911-x>
- Malécange, M., Sergheraert, R., Teulat, B., Mounier, E., Lothier, J., Sakr, S., 2023. Biostimulant Properties of Protein Hydrolysates: Recent Advances and Future Challenges. *Int J Mol Sci*. <https://doi.org/10.3390/ijms24119714>
- Malík, M., Velechovský, J., Praus, L., Janatová, A., Kahánková, Z., Klouček, P., Tlustoš, P., 2022. Amino Acid Supplementation as a Biostimulant in Medical Cannabis (*Cannabis sativa* L.) Plant Nutrition. *Front Plant Sci* 13. <https://doi.org/10.3389/fpls.2022.868350>
- Mattarozzi, M., Di Zinno, J., Montanini, B., Manfredi, M., Marengo, E., Fornasier, F., Ferrarini, A., Careri, M., Visioli, G., 2020. Biostimulants applied to maize seeds modulate the enzymatic activity and metaproteome of the rhizosphere. *Applied Soil Ecology* 148. <https://doi.org/10.1016/j.apsoil.2019.103480>
- Meena, D.C., Birthal, P.S., Kumara, T.M.K., 2025. Biostimulants for sustainable development of agriculture: a bibliometric content analysis. *Discover Agriculture* 3. <https://doi.org/10.1007/s44279-024-00149-5>

- Mironenko, G.A., Zagorskii, I.A., Bystrova, N.A., Kochetkov, K.A., 2022. The Effect of a Biostimulant Based on a Protein Hydrolysate of Rainbow Trout (*Oncorhynchus mykiss*) on the Growth and Yield of Wheat (*Triticum aestivum* L.). *Molecules* 27. <https://doi.org/10.3390/molecules27196663>
- Monterisi, S., Garcia-Perez, P., Buffagni, V., Zuluaga, M.Y.A., Ciriello, M., Formisano, L., El-Nakhel, C., Cardarelli, M., Colla, G., Roupael, Y., Cristofano, F., Cesco, S., Lucini, L., Pii, Y., 2024a. Unravelling the biostimulant activity of a protein hydrolysate in lettuce plants under optimal and low N availability: a multi-omics approach. *Physiol Plant* 176. <https://doi.org/10.1111/ppl.14357>
- Monterisi, S., Garcia-Perez, P., Buffagni, V., Zuluaga, M.Y.A., Ciriello, M., Formisano, L., El-Nakhel, C., Cardarelli, M., Colla, G., Roupael, Y., Cristofano, F., Cesco, S., Lucini, L., Pii, Y., 2024b. Unravelling the biostimulant activity of a protein hydrolysate in lettuce plants under optimal and low N availability: a multi-omics approach. *Physiol Plant* 176. <https://doi.org/10.1111/ppl.14357>
- Mpai, S., Mokganya, L.M., Raphoko, L., Masoko, P., Ndhlala, A.R., 2022. Untargeted metabolites and chemometric approach to elucidate the response of growth and yield attributes on different concentrations of an amino acid based biostimulant in two lettuce cultivars. *Sci Hortic* 306. <https://doi.org/10.1016/j.scienta.2022.111478>
- Mponda, S.Y., Kim, J.K., 2023. Sustainable, quality biofertilizer produced from Nile perch (*Lates niloticus*) wastewater using three *Bacillus* species: Demonstration in barley hydroponics. *J Environ Chem Eng* 11. <https://doi.org/10.1016/j.jece.2023.110037>
- Osman, A., Merwad, A.R.M., Mohamed, A.H., Sitohy, M., 2021a. Foliar spray with pepsin- and papain-whey protein hydrolysates promotes the productivity of pea plants cultivated in clay loam soil. *Molecules* 26. <https://doi.org/10.3390/molecules26092805>

- Osman, A., Merwad, A.R.M., Mohamed, A.H., Sitohy, M., 2021b. Foliar spray with pepsin- and papain-whey protein hydrolysates promotes the productivity of pea plants cultivated in clay loam soil. *Molecules* 26. <https://doi.org/10.3390/molecules26092805>
- Pan, W., Tang, S., Zhou, J., Liu, M., Xu, M., Kuzyakov, Y., Ma, Q., Wu, L., 2022. Plant–microbial competition for amino acids depends on soil acidity and the microbial community. *Plant Soil* 475, 457–471. <https://doi.org/10.1007/s11104-022-05381-w>
- Parađiković, N., Teklić, T., Zeljković, S., Lisjak, M., Špoljarević, M., 2019. Biostimulants research in some horticultural plant species—A review. *Food Energy Secur.* <https://doi.org/10.1002/fes3.162>
- Paul, K., Sorrentino, M., Lucini, L., Rouphael, Y., Cardarelli, M., Bonini, P., Miras Moreno, M.B., Reynaud, H., Canaguier, R., Trtílek, M., Panzarová, K., Colla, G., 2019. A combined phenotypic and metabolomic approach for elucidating the biostimulant action of a plant-derived protein hydrolysate on tomato grown under limited water availability. *Front Plant Sci* 10. <https://doi.org/10.3389/fpls.2019.00493>
- Pérez-Aguilar, H., Hernández-Fernández, C., Arán-Ais, F., 2024. Protein recovery from animal by-product wastewater rich in protein for application as biostimulant in agriculture. *Journal of Water Process Engineering* 66. <https://doi.org/10.1016/j.jwpe.2024.106120>
- Pérez-Aguilar, H., Lacruz-Asaro, M., Ruzafa-Silvestre, C., Arán-Ais, F., 2023. Protein recovery from wastewater animal processing by-products of rendering plants for biostimulant applications in agriculture. *Sustain Chem Pharm* 32. <https://doi.org/10.1016/j.scp.2023.101009>
- Petrozza, A., Santaniello, A., Summerer, S., Di Tommaso, G., Di Tommaso, D., Paparelli, E., Piaggese, A., Perata, P., Cellini, F., 2014. Physiological responses to Megafol®

- treatments in tomato plants under drought stress: A phenomic and molecular approach. *Sci Hortic* 174, 185–192. <https://doi.org/10.1016/j.scienta.2014.05.023>
- Polo, J., Mata, P., 2018. Evaluation of a biostimulant (Pepton) based in enzymatic hydrolyzed animal protein in comparison to seaweed extracts on root development, vegetative growth, flowering, and yield of gold cherry tomatoes grown under low stress ambient field conditions. *Front Plant Sci* 8. <https://doi.org/10.3389/fpls.2017.02261>
- Popko, M., Michalak, I., Wilk, R., Gramza, M., Chojnacka, K., Górecki, H., 2018. Effect of the new plant growth biostimulants based on amino acids on yield and grain quality of winter wheat. *Molecules* 23. <https://doi.org/10.3390/molecules23020470>
- Qiao, Q., Zhang, Jingxia, Ma, C., Wang, F., Chen, Y., Zhang, C., Zhang, H., Zhang, Jun, 2019. Characterization and variation of the rhizosphere fungal community structure of cultivated tetraploid cotton. *PLoS One* 14. <https://doi.org/10.1371/journal.pone.0207903>
- Ranocha, P., Dima, O., Nagy, R., Felten, J., Corratge-Faillie, C., Novak, O., Morreel, K., Lacombe, B., Martinez, Y., Pfrunder, S., Jin, X., Renou, J.P., Thibaud, J.B., Ljung, K., Fischer, U., Martinoia, E., Boerjan, W., Goffner, D., 2013. *Arabidopsis* WAT1 is a vacuolar auxin transport facilitator required for auxin homeostasis. *Nat Commun* 4. <https://doi.org/10.1038/ncomms3625>
- Rouphael, Y., Cardarelli, M., Bonini, P., Colla, G., 2017. Synergistic action of a microbial-based biostimulant and a plant derived-protein hydrolysate enhances lettuce tolerance to alkalinity and salinity. *Front Plant Sci* 8. <https://doi.org/10.3389/fpls.2017.00131>
- Rouphael, Y., Carillo, P., Garcia-Perez, P., Cardarelli, M., Senizza, B., Miras-Moreno, B., Colla, G., Lucini, L., 2022. Plant biostimulants from seaweeds or vegetal proteins enhance the salinity tolerance in greenhouse lettuce by modulating plant metabolism in a distinctive manner. *Sci Hortic* 305. <https://doi.org/10.1016/j.scienta.2022.111368>

Santi, C., Zamboni, A., Varanini, Z., Pandolfini, T., 2017. Growth stimulatory effects and genome-wide transcriptional changes produced by protein hydrolysates in maize seedlings. *Front Plant Sci* 8. <https://doi.org/10.3389/fpls.2017.00433>

Sestili, F., Roupael, Y., Cardarelli, M., Pucci, A., Bonini, P., Canaguier, R., Colla, G., 2018a. Protein hydrolysate stimulates growth in tomato coupled with N-dependent gene expression involved in N assimilation. *Front Plant Sci* 9. <https://doi.org/10.3389/fpls.2018.01233>

Sestili, F., Roupael, Y., Cardarelli, M., Pucci, A., Bonini, P., Canaguier, R., Colla, G., 2018b. Protein hydrolysate stimulates growth in tomato coupled with N-dependent gene expression involved in N assimilation. *Front Plant Sci* 9. <https://doi.org/10.3389/fpls.2018.01233>

Shestakova, A., Timorshina, S., Osmolovskiy, A., 2021. Biodegradation of keratin-rich husbandry waste as a path to sustainable agriculture. *Sustainability (Switzerland)*. <https://doi.org/10.3390/su13168691>

Sobucki, L., Ramos, R.F., Gubiani, E., Brunetto, G., Kaiser, D.R., Daroit, D.J., 2019. Feather hydrolysate as a promising nitrogen-rich fertilizer for greenhouse lettuce cultivation. *International Journal of Recycling of Organic Waste in Agriculture* 8, 493–499. <https://doi.org/10.1007/s40093-019-0281-7>

Soppelsa, S., Kelderer, M., Casera, C., Bassi, M., Robatscher, P., Andreotti, C., 2018. Use of biostimulants for organic apple production: effects on tree growth, yield, and fruit quality at harvest and during storage. *Front Plant Sci* 9. <https://doi.org/10.3389/fpls.2018.01342>

- Sytar, O., Kumar, A., Latowski, D., Kuczynska, P., Strzałka, K., Prasad, M.N.V., 2013. Heavy metal-induced oxidative damage, defense reactions, and detoxification mechanisms in plants. *Acta Physiol Plant*. <https://doi.org/10.1007/s11738-012-1169-6>
- Tanou, G., Ziogas, V., Molassiotis, A., 2017. Foliar nutrition, biostimulants and prime-like dynamics in fruit tree physiology: New insights on an old topic. *Front Plant Sci* 8. <https://doi.org/10.3389/fpls.2017.00075>
- Tegeder, M., 2014. Transporters involved in source to sink partitioning of amino acids and ureides: Opportunities for crop improvement. *J Exp Bot*. <https://doi.org/10.1093/jxb/eru012>
- Tegeder, M., Masclaux-Daubresse, C., 2018. Source and sink mechanisms of nitrogen transport and use. *New Phytologist*. <https://doi.org/10.1111/nph.14876>
- Teixeira, W.F., Fagan, E.B., Soares, L.H., Soares, J.N., Reichardt, K., Neto, D.D., 2018. Seed and foliar application of amino acids improve variables of nitrogen metabolism and productivity in soybean crop. *Front Plant Sci* 9. <https://doi.org/10.3389/fpls.2018.00396>
- Van Oosten, M.J., Pepe, O., De Pascale, S., Silletti, S., Maggio, A., 2017. The role of biostimulants and bioeffectors as alleviators of abiotic stress in crop plants. *Chemical and Biological Technologies in Agriculture*. <https://doi.org/10.1186/s40538-017-0089-5>
- Vaseva, I.I., Simova-Stoilova, L., Kostadinova, A., Yuperlieva-Mateeva, B., Karakicheva, T., Vassileva, V., 2022. Heat-Stress-Mitigating Effects of a Protein-Hydrolysate-Based Biostimulant Are Linked to Changes in Protease, DHN, and HSP Gene Expression in Maize. *Agronomy* 12. <https://doi.org/10.3390/agronomy12051127>
- Viebahn, M., Doornbos, R., Wernars, K., Van Loon, L.C., Smit, E., Bakker, P.A.H.M., 2005. Ascomycete communities in the rhizosphere of field-grown wheat are not affected by

- introductions of genetically modified *Pseudomonas putida* WCS358r. Environ Microbiol 7, 1775–1785. <https://doi.org/10.1111/j.1462-2920.2005.00783.x>
- Visconti, F., de Paz, J.M., Bonet, L., Jordà, M., Quiñones, A., Intrigliolo, D.S., 2015. Effects of a commercial calcium protein hydrolysate on the salt tolerance of *Diospyros kaki* L. cv. “*Rojo Brillante*” grafted on *Diospyros lotus* L. Sci Hortic 185, 129–138. <https://doi.org/10.1016/j.scienta.2015.01.028>
- Wahab, A., Abdi, G., Saleem, M.H., Ali, B., Ullah, S., Shah, W., Mumtaz, S., Yasin, G., Muresan, C.C., Marc, R.A., 2022. Plants’ Physio-Biochemical and Phyto-Hormonal Responses to Alleviate the Adverse Effects of Drought Stress: A Comprehensive Review. Plants. <https://doi.org/10.3390/plants11131620>
- Wilson, H.T., Amirkhani, M., Taylor, A.G., 2018. Evaluation of gelatin as a biostimulant seed treatment to improve plant performance. Front Plant Sci 9. <https://doi.org/10.3389/fpls.2018.01006>
- Wise, K., Selby-Pham, J., Chai, X., Simovich, T., Gupta, S., Gill, H., 2024. Fertiliser supplementation with a biostimulant complex of fish hydrolysate, Aloe vera extract, and kelp alters cannabis root architecture to enhance nutrient uptake. Sci Hortic 323. <https://doi.org/10.1016/j.scienta.2023.112483>
- Xu, L., Geelen, D., 2018. Developing biostimulants from agro-food and industrial by-products. Front Plant Sci. <https://doi.org/10.3389/fpls.2018.01567>
- Yao, X., Nie, J., Bai, R., Sui, X., 2020. Amino acid transporters in plants: Identification and function. Plants. <https://doi.org/10.3390/plants9080972>

Yuan, D., Wu, X., Jiang, X., Gong, B., Gao, H., 2024. Types of Membrane Transporters and the Mechanisms of Interaction between Them and Reactive Oxygen Species in Plants.

Antioxidants. <https://doi.org/10.3390/antiox13020221>

Zhang, Y.M., Ye, D.X., Liu, Y., Zhang, X.Y., Zhou, Y.L., Zhang, L., Yang, X.L., 2023.

Peptides, new tools for plant protection in eco-agriculture. *Advanced Agrochem.*

<https://doi.org/10.1016/j.aac.2023.01.003>

Zhao, S., Thakur, N., Salama, E.S., Zhang, P., Zhang, L., Xing, X., Yue, J., Song, Z., Nan, L.,

Yujun, S., Li, X., 2022. Potential applications of protein-rich waste: Progress in energy management and material recovery. *Resour Conserv Recycl.*

<https://doi.org/10.1016/j.resconrec.2022.106315>

Zhou, H., Jia, S., Gao, Y., Li, X., Lin, Y., Yang, F., Ni, K., 2024. Characterization of

phyllosphere endophytic lactic acid bacteria reveals a potential novel route to enhance silage fermentation quality. *Commun Biol* 7. [https://doi.org/10.1038/s42003-024-05816-](https://doi.org/10.1038/s42003-024-05816-3)

3

Zulfiqar, F., Akram, N.A., Ashraf, M., 2020. Osmoprotection in plants under abiotic stresses:

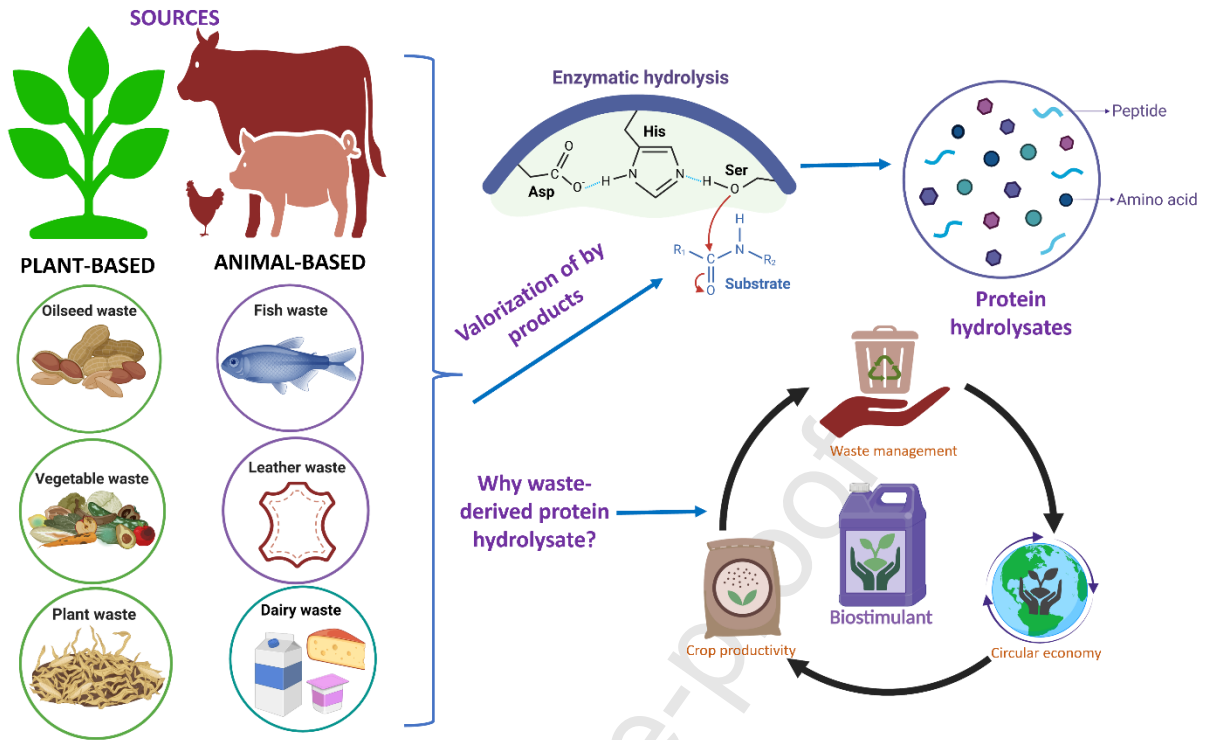
new insights into a classical phenomenon. *Planta*. [https://doi.org/10.1007/s00425-019-](https://doi.org/10.1007/s00425-019-03293-1)

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Graphical abstract



- Protein hydrolysates for agriculture are produced from plant and animal waste.
- Biostimulant effects are driven by a variety of peptides and amino acids.
- Plant growth and stress tolerance are enhanced by protein hydrolysates.
- Protein hydrolysates improve crop yields and nutrient uptake.
- Plant microbiome and molecular responses are modulated by protein hydrolysate.

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