

Plant Biostimulants

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Modern agriculture is being challenged by deteriorating edaphoclimatic conditions and increasing anthropogenic pressure. This necessitates the development of innovative crop production systems that can sustainably meet the demands of a growing world population while minimizing the environmental impact. The use of plant biostimulants is gaining ground as a safe and ecologically sound approach to improving crop yields.

agriculture

climate change

abiotic stress

reactive chemical species

plant biostimulants

higher plant-derived biostimulants

1. Introduction

One of the main challenges facing modern agriculture is finding a sustainable way of feeding the growing world population, which the United Nations estimates will increase by nearly 2 billion people over the next 30 years, considering the decrease in crop area ^[1]. The use of agrochemicals to boost food production is becoming increasingly unsustainable due to indiscriminate use, prompting stricter regulations. Moreover, there is growing consumer demand for ecological products, particularly since the COVID-19 pandemic. Products with “BIO” or “ECO” labels, indicating sustainable production systems free of agrochemicals, are regarded as healthier alternatives to conventionally produced foods ^[2]. The need to maintain product quality standards at the highest level is evidenced by the data provided by the Food and Agriculture Organization of the United Nations (FAO), which highlights that 90% of vitamin C and 60% of vitamin A consumed by the human population come from agricultural crops ^[3]. Therefore, it is important to guarantee both quality standards and food security of the population.

In the move toward sustainable agriculture, the Farm to Fork Strategy, included in the European Green Deal, has set a deadline of 2030 to reduce the use of chemical pesticides by 50% as well as soil nutrient losses by at least 50%, which in turn should cut the use of fertilizers by at least 20%, with the intention of allocating 25% of agricultural areas to organic farming ^{[4][5]}.

These limitations and reductions in the use of agrochemicals have been the consequence of years of their abuse in agriculture, facts that have manifested themselves in the deterioration of soils, along with the potential damage to human health and the environment. Among them, the uncontrolled use of nitrogen-rich fertilizers has triggered eutrophication processes, with consequent severe environmental damage, as well as nitrate accumulation in

plants, exceeding the regulation limits that allow their consumption. In fact, the nitrates present in these foods are metabolized into potentially carcinogenic compounds that are harmful to human health [6].

Another challenge facing modern agriculture is the diminishing availability of land for crop production due to climate change. Alterations in rainfall patterns and escalating global temperatures are leading to the aridification of arable lands, rendering them unprofitable for food production [7].

All these factors indicate there is an urgent need to develop more sustainable agricultural systems capable of providing for the growing population while minimizing the environmental impact. Overcoming the increasingly adverse anthropological and edaphoclimatic conditions that limit production performance is imperative. Moreover, the long processes of genetic improvement through breeding developed over the years in crops are reaching the limit of their potential. Given that improving crop tolerance to climate change by genetic modifications or in vitro selection takes years to accomplish, it is of paramount interest to search for alternative strategies with a more immediate impact [8].

2. Plant Biostimulants: An Emerging Ecological Alternative

The use of biostimulants among sustainable agricultural practices is gaining ground as a promising, safe, and ecological alternative to improving crop production performance.

Prior to the term “biostimulant”, the terms “biogenic stimulators” or “biogenetic stimulants” were used to refer to substances synthesized in tissues under stressful, but not lethal, conditions, which stimulated the vital reactions of the organism [8]. The term “biostimulant” was used for the first time in a research article by Russo and Berlyn published in 1991 [9]. These authors went on to define biostimulants in 1992 [10] as “non-nutritional products that may reduce fertilizer use and increase yield and resistance to water and temperature stresses”, also remarking that they “stimulate plant growth [when used] in relatively small amounts” [11].

Despite the recent exponential increase in research on biostimulants, controversy remained regarding their precise definition. Until official regulation by the European Union in 2019, the status of biostimulants differed between member states and they were marketed as biofertilizers in mixtures with nutritional elements [12][13]. Nevertheless, by 2019, a consensus on the key attributes of a biostimulant had been reached. These include the ability to act on plant homeostasis at low doses, improve plant growth, induce a more efficient use of nutrients and water, modulate abiotic stress response, and exert synergistic effects resulting from the combination of bioactive compounds [11]. Finally, Europe laid the foundations for the regulation of these products, with the publication of Regulation (EU) 2019/1009 of the European Parliament and of the Council of 5 June 2019, where biostimulants are defined as [14]:

“A plant biostimulant shall be an EU fertilising product the function of which is to stimulate plant nutrition processes independently of the product’s nutrient content with the sole aim of improving one or more of the following characteristics of the plant or the plant rhizosphere:

- (a) nutrient use efficiency,
- (b) tolerance to abiotic stress,
- (c) quality traits, or
- (d) availability of confined nutrients in the soil or rhizosphere."

Accordingly, it was established that the main difference between a biofertilizer and a biostimulant is that a biofertilizer participates in the provision of nutrients to the plant, whereas biostimulants favor nutrient acquisition [15]. Furthermore, in contrast to plant defense elicitors, which provide a protection against biotic stress by inducing systemic acquired resistance, biostimulants act by providing tolerance to abiotic stress [16].

The lack of a clear definition due to the appearance of the concept of biostimulants in the scientific world has compromised the way of properly classifying them. The wide diversity of biostimulants, in both origin and composition, as well as the physiological functions triggered by their application, has complicated their classification, although the scientific community has widely accepted the proposal of Patrick du Jardin in 2015 [17], who divided biostimulants into seven categories. Humic substances, which include humic and fulvic acids, correspond to heterogeneous organic molecules resulting from the decomposition of organic remains in the soil. Protein hydrolysates (PHs) are mixtures of variable proportions of free amino acids, oligopeptides, and polypeptides, obtained through the chemical and enzymatic hydrolysis of proteins from various sources, both animal and plant, generally obtained from industrial by-products and waste. Seaweed and botanical extracts are variable and heterogeneous mixtures that, depending on the origin of the extract, include complex polysaccharides, phenolic compounds, and hormones, among others. Biopolymers, which include a wide diversity of molecules of highly variable size and characteristics, are obtained by extraction or industrial synthesis. Inorganic compounds correspond to beneficial elements, mainly Al, cobalt (Co), Na, selenium (Se), and silicon (Si), present as different inorganic salts. Beneficial fungi and bacteria constitute a very broad and varied group that include symbiotic microorganisms and plant growth promoters [17].

Despite their heterogeneity, biostimulants are generally applied in three different ways: through seed priming, application to soil (or to nutrient solution for hydroponic crops), and in foliar spray (**Figure 1**). In seed priming, the seeds are soaked in a solution containing the biostimulant to enable ingredient penetration. In the case of soil application, the product effectiveness depends on adequate soaking, while the application in hydroponic cultivation is more straightforward, with the desired concentration simply being added to the nutrient solution. In foliar spraying, the biostimulant is applied in solution when the seedling reaches a minimum age, typically after the generation of several true leaves. The frequency of application varies according to the biostimulant used [18]. The efficacy of a biostimulant hinges on the penetration of active ingredients into the seeds, roots, or leaves, and their assimilation is based on factors such as molecular structure, particle size, and solubility. Additives are commonly used to optimize solubility, absorption capacity, and penetration into the plant material [19].

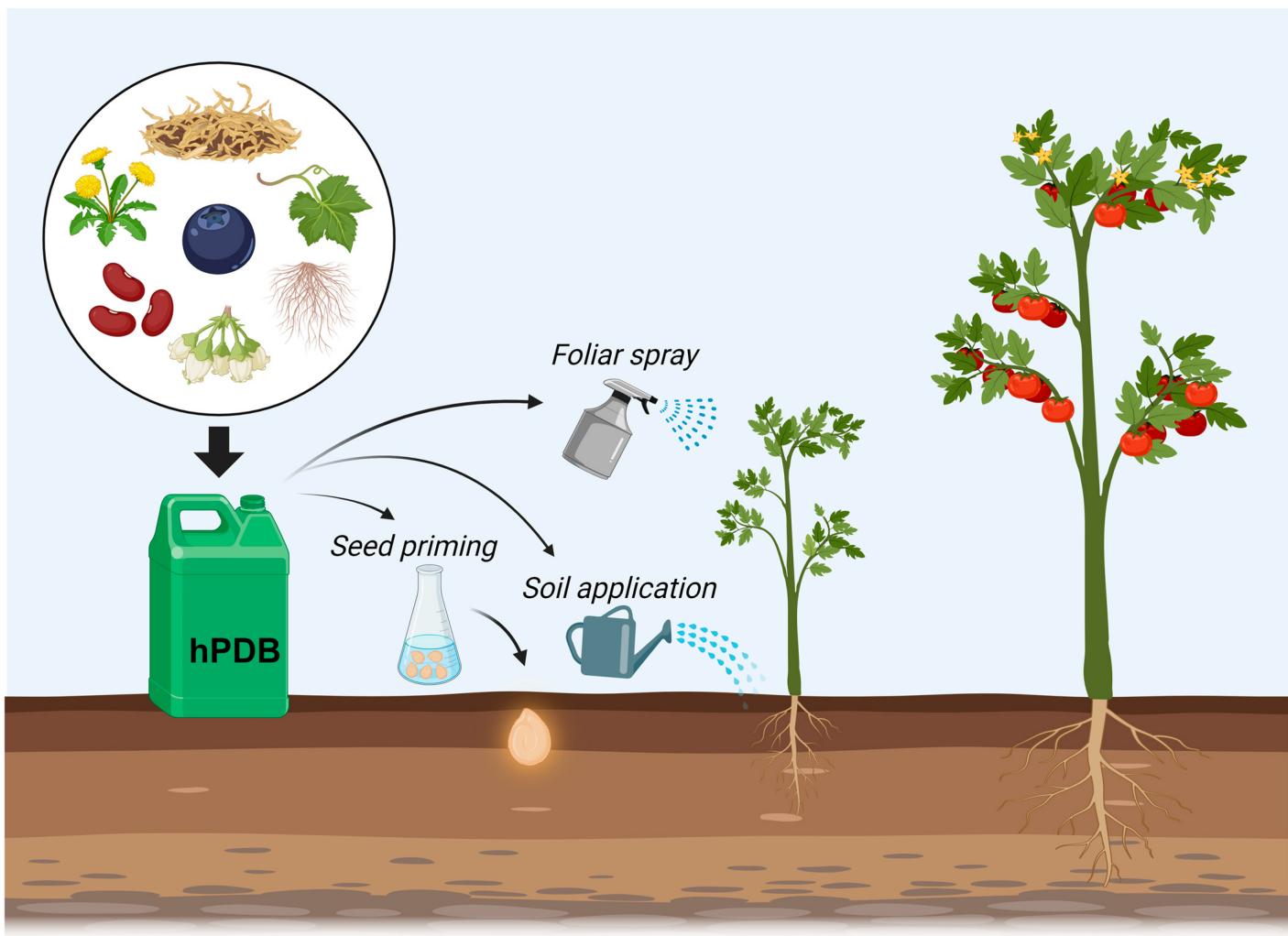


Figure 1. hPDB main sources and application method. Created with [BioRender.com](https://biorender.com), accessed on 31 January 2024.

Biostimulants from natural sources have attracted attention for their sustainability, ecological advantages, and biodegradability, ensuring minimum or null environmental impact. Among them, higher plant-derived biostimulants (hPDBs) stand out, above animal-derived biostimulants (ADB), because their sustainability and profitability are superior. In fact, the production of ADBs generates more CO₂ emissions (+57%) and consumes more energy (+26%) and water [20]. Furthermore, animal-derived by-products, the source of most ADBs, represent a potential risk for the consumer through disease transmission [21]. Regarding microorganism-derived biostimulants, which have also attracted a lot of attention, there is controversy about their application, because some microbial products are not normally present in agricultural fields [8].

Researchers define the term hPDBs as group of biostimulants that gather PHs, extracts from plant by-products, whole plants or specific organs, purified metabolites, and cell cultures derived from higher plants, included in the Tracheophyta phylum (Figure 1). Biostimulants, by definition, are effective in inducing physiological processes in plants that improve their growth and acclimation to stress, concluding in an increase in crop yield. Furthermore, the generation of abundant plant by-products after obtaining the marketable and consumable parts of the crop is very

common, so they can be revalued and used to increase crop production and profitability while reducing the environmental impact of waste disposal [22]. hPDBs are of great interest due to the enormous variability of biomolecules that compose them, from peptides to specialized metabolites, which is the reason they can potentially induce a multitude of physiological processes that lead to an increased crop yield [18].

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