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Review

Scientific Advances in Biostimulation Reported in the 5th Biostimulant World Congress

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Abstract: Biostimulants are agronomic products that have become highly important in agriculture, as they are formulated with substances capable of stimulating physiological and biochemical processes in plants that help them adapt to different detrimental environmental conditions such as drought, salinity, high temperatures, nutritional deficiencies, etc. in such a way that the crops, under these conditions, maintain a good agronomic yield and quality of harvest. Every two years, the International Congress on Biostimulants is held with the aim of publicizing the most innovative and recent advances in every relevant type of product: new active ingredients, modes of action, cultivation protocols, test platforms and phenotyping, use of analytical omics tools, etc. In December 2021, the 5th World Congress on Biostimulants was held in Miami (Florida, USA). This publication summarizes the most relevant results that were presented at this congress, in which biostimulants formulated with algae extracts and amino acids stood out in a number of presentations. The various studies presented were carried out on diverse crops such as apple, blackberry, carrot, celery, cherry, corn, grape (table and wine), olive, pear, pepper, potato, rapeseed, spinach, sunflower, soybean, tomato, and wheat. The future lines of the new generation of biostimulants were also marked.

Keywords: active principle; agrochemical products; horticultural and fruit crops; natural extracts; tolerance to abiotic stresses

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1. Introduction

A biostimulant is an agrochemical product formulated with mixtures of natural substances and/or microorganisms which, when applied to plants, can improve the efficiency of mineral nutrition, tolerance to abiotic stress (salinity, drought, high temperatures, heavy metals, etc.), and biotic and/or crop yield, or enhance quality characteristics, regardless of its content in essential mineral nutrients for plants. In general, nine categories of substances that act as biostimulants have been defined: (i) humic substances; (ii) complex organic materials (obtained from agro-industrial and urban waste, sewage sludge extracts, compost, and manure); (iii) beneficial chemical elements (Al, Co, Na, Se, and Si); (iv) inorganic salts, including phosphite; (v) algae extracts (brown, red, and green macroalgae); (vi) derivatives of chitin and chitosan; (vii) antiperspirants (kaolin and polyacrylamide); (viii) free amino acids and N-containing substances (peptides, polyamines, and betaines); and (ix) plant growth promoting rhizobacteria (PGPR), arbuscular mycorrhizal fungi (AMF), and *Trichoderma* spp. Biostimulants are usually able to improve vigor, stimulate vegetative growth, improve the absorption and distribution of nutrients within the plant, increase the antioxidant capacity, and improve tolerance to biotic and abiotic stresses, consequently improving the yield of the plant and the quality of the fruit. Such is the importance of these agrochemical products in agriculture and in rural society that, in the European Union, the term biostimulant appears in its regulation (EU) 2019/1009, classifying them as microbial and non-microbial biostimulants.

The International Congress on Biostimulants is organized every two years with the aim of publicizing the new advances in R+D that are being carried out for the formulation of these agrochemical products. Every year sees a new record in the number of attendees, companies, and participating research centers. The 5th International Congress of Biostimulants was held in Miami in 2021 with the participation of 770 delegates and the presentation of more than 100 articles, including conferences and panels. The present publication highlights the most important scientific aspects of this congress, in which the results of biostimulants formulated with microorganisms, algae extracts, amino acids and hydrolyzed proteins, molecular complexes, metalloids, phospholipids, growth-regulating molecules, and natural extracts were presented.

2. Activate Matter That Is Part of Biostimulant Products

2.1. Algae

AlgaEnergy [1–4], a world-leading Spanish company in microalgae biotechnology, presented the results of its microalgae application trials on olives, cherry, spinach, rice, and wheat. These extracts improved the production and quality of the olive tree harvest (increased the content of monosaturated and polyunsaturated fatty acids in the oil), increased the dry matter content and the concentration of chlorophylls in the spinach crop, and increased the weight and the texture of cherry fruits. In addition, the company showed that the application of a microalgae extract with mycorrhizae managed to increase the production of rice and wheat in India as compared with the production from conventional crop management.

Agricola2000 scpa [5] showed that a Tradecorp biostimulant, formulated based on *Ascophyllum nodosum* extracts, increased the tolerance to heat stress (37 °C) of *Diplotaxis tenuifolia* plants. The transcriptomic study revealed that its application activated genes related to heat stress and the antioxidant system of plants.

Brandon Bioscience [6], a company located in the south of Ireland, reported at the congress that the application of a product formulated with *A. nodosum* and containing the molecular complex PSI-759 reduced the loss of seeds in rapeseed and soybean through plant regulation signaling processes.

Ficosterra [7], a Spanish marine biotechnology company, demonstrated that the application of the products Ficosagro® and Cystium-KA® (both containing algae extracts, such as *Macrocystis pyrifera*, and microorganisms) increased the production of horticultural crops; at the same time, the contribution of conventional N and P fertilizers was reduced.

Green Has [8], also a Spanish company specializing in the nutrition sector, presented the results of its Expandoa® product. This product is formulated based on algae and yeast extracts, and favors the production, growth, and nutritional quality of tomato fruits. Its mode of action is associated with the positive regulation of genes involved in the synthesis of chlorophylls and carotenoids. It also presented the products Eranthis® [9] and Algaren Twin® [10]. Regarding Eranthis®, a product formulated with brown marine algae (*A. nodosum, Laminaria digitata*) and yeast extracts, it was shown that it provides plants (lettuce, tomato, and zucchini) with a large number of compounds such as amino acids, peptides, glycine betaine, mannitol, alginates, fucodians, and polyphenols. These compounds stimulate the physiological processes of plants and provide strong protection against oxidative stress caused by different abiotic stresses. The great anti-stress capacity of this product was verified in vegetables, perennial crops, and cereals. Regarding Algaren Twin®, a product formulated with extracts from the algae *Ecklonia maxima* that contains a large amount of hormones with a physiological function in plants such as cytokinins, auxins, gibberellins, abscisic acid, and ethylene, it was shown that rooting, flowering, and fruiting were established in plants both under normal conditions and under climate change scenarios through the activation of genes and metabolic pathways specific to plants.

The application of Expando [11] was tested at the University of Turin to increase the nutritional properties of *Prunus persica* fruits. This product is composed of algae and yeast extracts. Its application increase the concentration of various phenolic compounds

in the skin and pulp of the fruits; specifically increasing flavon-3-ols, anthocyanins, and flavonoids. This increase made the fruits have a higher antioxidant capacity, an aspect that is highly valued by consumers.

RPC Kvadrat [12] is a Ukrainian company that presented its biostimulant Quantum®, formulated with extracts and amino acids from the algae *A. nodosum*. When this biostimulant was applied to wheat plants, it was observed that the production increased by 22% due to the stimulation of the antioxidant system of the plants, specifically, via the enzymes ascorbate peroxidase and superoxide dismutase, which reduce the concentration of reactive oxygen species.

St Malo Biological Laboratory [13], directed by Dr. Guiboileau, showed that the application of biostimulants formulated with algae extracts can increase the effective use of nitrogen of crops due to the overexpression of different genes related to nitrogen metabolism at the level of both absorption and assimilation. Thus, the application of these extracts increased the nitrogen content in tomato, wheat, and corn plants.

Brandon Bioscience [14], with the help of Dr. Lukaz Lagowski, revealed the mode of action by which biostimulant products formulated with extracts from the *A. nodosum* seaweed can increase the efficient use of nitrogen in agricultural systems. Genomic studies have shown that these biostimulants can induce the expression of genes related to the absorption of nitrate by the roots and increase the enzymatic activity of proteins related to nitrogen assimilation.

Algaia [15], a French company dedicated to the processing of algae, showed a novel technique that allows them to extract compounds from algae with a high capacity to stimulate the growth of beneficial microorganisms for plants in the soil through the secretion of growth-promoting compounds.

2.2. Amino Acids

Agricola2000 [16] showed that the application of a product formulated with amino acids of animal origin, through bacterial fermentation, increased the tolerance of *Diplotaxis tenuifolia* and *Arabidopsis thaliana* plants to heat stress (37 °C). The transcriptomic study revealed that its application activated genes related to heat stress and the antioxidant system of plants.

BCF Life Science [17], a French company specializing in the extraction of natural amino acids and their derivatives, presented Leafamine®, a biostimulant formulated with amino acids (hydrolyzed proteins of animal origin) that stimulated the action of soil microorganisms, which led to an increase in the production and concentration of amino acids in the fruits of a strawberry crop. It was also reported that this product can increase the production of irrigated lettuce in good conditions or in situations of water deficit. The mechanisms involved in these responses were related to the accumulation of osmolytes and antioxidant molecules.

CEBAS-CSIC and Atlántica Agrícola [18], in one of the many examples of knowledge transfer between companies and research centers in Spain, presented the results obtained in a study carried out on tomato plants, where the importance of determining the synergistic effects that exist between mixtures of free amino acids was described. The application of a mixture of amino acids to tomato plants increased net CO_2 assimilation and total soluble sugars and deactivated reactive oxygen species in plants grown with saline waters.

Bioibérica [19] presented the product TerraminA®Pro, formulated with amino acids. It is capable of activating the soil microbiota, which stimulates the absorption of nutrients by plants, and therefore, the contribution of nitrogenous fertilizers to crops can be reduced. In addition, it increases resistance to abiotic stresses.

The Greek company EVYP LLP [20] presented Amino16®, a formulation with protein hydrolysates that contain a large amount of amino acids. Its application increased the grain harvested in wheat cultivation while reducing the quantity of conventional nitrogen fertilizers used, thus avoiding the accumulation of nitrates in the soil and therefore reducing environmental pollution.

Fertinagro [21] is a Spanish company from the Térvalis Group, which demonstrated how the application of a mixture of 18 amino acids of natural origin increased the rate of root growth, favoring the general development of wheat and corn plants. The mechanisms involved in this response included an increase in the concentration of chlorophylls, saving time and energy by providing already-synthesized products, and an increase in the microbial biomass in the soil.

Promisol [22], a company from the northwest of Spain, presented the results of an experiment in which the effects of the application of a biostimulant product made with amino acids and algae extracts enriched with polyphenols were verified. The results showed that this product increases salinity tolerance by reducing H_2O_2 levels, Na concentration, and oxidative stress in the leaves.

Sensient Technologies [23] showed that the application of a product based on an animal protein hydrolyzate, BLOOMGENIXTM, in tomato, soybean, and lettuce plants improved the agronomic behavior of these crops in situations of abiotic stress by increasing the absorption of nutrients and stimulating the defense responses against these stresses.

Via vegetale [24], a French company, presented a biostimulant formulated with an organo-mineral complex containing amino acids, while also being rich in nitrogen (urea/ammonium/nitrate). The application of this product accelerated the senescence processes and the remobilization of nitrogen from the root to the grain, increasing the grain yield per plant, the nitrogen harvest index, and the nitrogen use efficiency. This trial was carried out in six different locations in Normandy, France, with the beneficial effect of the biostimulant observed in all of them.

Nixe Laboratories [25], presented the effects of the application of a biostimulant (Trainer®) made with defatted soybean protein hydrolysates to tomato plants and *Arabidopsis thaliana*. The results showed that the application of this product increased photosynthesis, hormonal balance, assimilation of macronutrients, and resistance to abiotic stresses (salinity, heat, and drought) in such a way that the growth of these plants was improved in adverse environmental conditions.

The Italian university (Catholic University of the Sacred Heart) [26] demonstrated how the application of glycine betaine (Vegetable B60, EDF Man) to tomato plants improved their tolerance to drought. Due to the fact that the application of this molecule favored the detoxification of reactive oxygen species, the photosynthetic machinery was stabilized, lipid synthesis increased, and the thickness of the leaves also increased.

Dr. Guiseppe Colla [27] from the University of Tucsia presented his studies, within the framework of the PHOBOS project, on the benefits of applying protein hydrolysates to crops. These compounds are capable of promoting root growth mainly due to the action of specific peptides and amino acids on the synthesis of indoleacetic acid.

2.3. Organic Acids

Bio Huma Netic, Inc. [28] presented a humic acid product extracted from shale ore. This product was applied to MicroTom plants subjected to nutrient deficiency, which produced growth and fruit yield similar to plants grown without NPK nutrient deficiency. The mode of action of this product seems to be related to the activation of flavonoids, quinones, and alkaloids, and the increase of antioxidant and antimicrobial activity. Omics analytical techniques such as FTICR-MS and ^{13}C-NMR were used to investigate the mode of action of this biostimulant.

Dr. Jamal Javanmardi [29] demonstrated that the application of a tea compost and vermicompost produced benefits in tomato crops. The modes of action involved in this effect were related to the fact that this vermicompost has extracellular compounds from organisms that act as growth promoters and also has a high concentration of nutrients available to plants.

FBSciences, Inc. [30] demonstrated how the application of a biostimulant, made with natural organic matter, to maize plants stimulated root growth faster and deeper in the soil profile as compared to untreated plants, which favored the final production of the crop.

Fertinagro [31] demonstrated how seeds coated with potassium phytate had better phosphorus nutrition, which favored a higher rate and speed of germination in sunflower, corn, and wheat crops. During the development of the crop, it also had beneficial effects on the development of the root and shoot, while at the same time providing savings on phosphorous fertilizers.

Iñesta Group [32] presented its product Bioest-24®, formulated from waste from the food industry, which is capable of increasing tolerance to cold and other stresses due to the activation of the plant's synthesis of proteins and phenolic compounds and the reduction of oxidative stress. The beneficial effects of this product were tested in in vitro barley cell cultures and in a field experiment with lettuce plants.

Innovak Global [33] presented Rhizoxfera TS®, a product formulated with phenolic substances whose application to seeds is capable of stimulating the symbiotic process between the root and nitrogen-fixing bacteria. The test was carried out on soybeans by the application of the product to the seeds and their inoculation with *Bradyrhizobium japonicum*. Treated plants grown without nitrogen application increased the nodulation rate (i.e., the number of nodules) and decreased the nodulation time, which favored grain production by the plants. The mode of action was described as an overexpression of the ACO1 gene, responsible for ethylene biosynthesis related to the germination process, and of the PIN 1 gene, involved in auxin transport and root development.

Tradecorp [34] presented Biimore®, a new biostimulant formulated from a biological fermentation process of food waste rich in sugars, which contains more than 200 synergistic compounds. One of its most interesting characteristics is that it is applied in ultra-low doses, having effects on the primary and secondary metabolism of plants.

2.4. Metalloids

Selenium [35]. Dr. Francisco García Sánchez, researcher at CEBAS-CSIC, demonstrated that the application of this metalloid to pepper plants in the form of selenate via the root improves the vegetative growth of pepper plants grown with water containing a high Cd concentration by reducing both its accumulation in the leaves and its toxicity to plants.

Titanium [36]. Intermag scientists showed data on how the application of organic titanium compounds could improve the pollination rates in apple, blackberry, and tomato plants. These scientists hypothesized that the application of these compounds increased the expression of genes that determine stamen and carpel development, pollen germination, and pollen tube growth and those that are involved in gibberellin biosynthesis; these compounds also decreased the expression of genes encoding the repressor proteins of the gibberellins signaling pathway (DELLA GAI protein).

Vanadium [37]. A trial that studied the foliar or root application of organic vanadium compounds revealed their beneficial effects on root growth rate in celery and carrot plants. The mode of action of these elements, when applied foliarly, was related to an overexpression of genes involved in the synthesis and regulation of phytohormones responsible for root growth and development and with the increased expression of genes involved in the biosynthesis, transport, and accumulation of storage substances, such as starch and sucrose. In addition, vanadium decreased the expression of the gene responsible for the production of polygalacturonase, an enzyme responsible for cell-wall breakdown and sugar degradation.

Silica [38]. A foliarly-applied silica-rich product had beneficial effects on wheat, corn, and apple plants grown under different stresses. The modes of action of this compound in tomato were an increase in the expression of genes related to the production of PR proteins, cellulose, and xyloglucan synthesis and a decrease in ABA gene expression. Furthermore, in drought and cold stress, protein detoxification gene overexpression also increased.

2.5. Products with Microorganisms

The application of an inoculum from *Bacillus amyloliquefaciens* (bacteria) [39] to a potato crop improved its performance. The author of this work, Dr. Alberto Acedo from Biome

Makers, concluded that the structure of the soil fungal community could be used as a biomarker of the final crop production, since both parameters are directly related.

The company Agrauxine [40] presented Fertispring®, a product formulated with inactive yeasts that activate soil microflora, fungi, and bacteria, which was created to help mineralize organic fertilizers in such a way that nutrients such as nitrogen are easier to assimilate by the plants. This same company also showed Moka® [41], a product with yeast extracts and other compounds such as vitamins and amino acids, whose aim is to simulate plant metabolism and improve fruit quality (texture, color, size, etc.). It was tested on table grapes and vinification.

The Italian company Agri 2000 Net Srl [42] demonstrated how the EXELGROW® product favored the ripening of table grapes in regions where the climate hinders this process.

Agricola 2000 [43] also presented a biostimulant formulated with yeast extract that increased nitrogen use efficiency (uptake and assimilation) in a trial carried out with bean plants.

TradecorpTM [44] presented a product formulated with bacterial fermentation extracts that increased the tolerance of *Arabidopsis thaliana* plants to heat stress (37 °C). The transcriptomic study revealed that its application activated genes related to heat stress and the antioxidant system of plants.

Products containing microorganisms have the problem that sometimes the packaging conditions cause the cells to lose their viability, thus reducing the effects for which they were formulated. Dr. Rebecca Willian-Wagner reported that low water vapor transmission packaged products improved the stability of lyophilized products formulated with microbial cells [45]. In addition, the gas permeability of the packaging affected the stability of microbial cells in liquid formulations. Therefore, it is necessary to improve and optimize the packaging of these products.

2.6. Natural Extracts

Fertinagro [46] studied the application of a natural extract produced by the *Metschnikowia pulcherrima* yeast in combination with a natural biostimulant that is capable of inhibiting urease activity and stimulating root growth. This combined application favored ammonium uptake by plants and reduced nitrogen leaching in the soil. This was demonstrated in forage crops, maize, and horticultural crops.

Within the project Life18/ENV/NL/000043 Plant for Plants (P4P) [47], Dr. Cristina Saduro presented data from a trial with a biostimulant that was formulated with botanical extracts and created to increase the tolerance of tomato plants to drought, that sought to establish the mode of action of said biostimulant. The plants under drought stress treated with the biostimulant product showed an increase in glucosinolates, conjugated polyamines, phytoalexins, brassinosteroids, and ascorbate, while phenylpropanoids were down-regulated. Tests were also carried out in this project to check whether this type of biostimulant could increase the effective use of phosphorus. For this, experiments were carried out on apple trees, in which it was observed that its application increased production, color, total soluble sugars, and firmness of the fruits. The results were also confirmed in strawberries.

2.7. Growth Regulators

Daminozide and paclobutrazol [48]. Dr. Alfosea-Simón from the CEBAS-CSIC studied how Petunias, Pelargonium, and Menthas ornamental plants treated with a foliar application of these growth-regulating compounds increased their life cycle during the transport phase. Specifically, it is at this time (transport phase) when the plants suffer greater stress due to drought, darkness, and low temperatures.

Brassinosteroids [49]. Dr. Robert Kremer from the University of Missouri presented a study on the application of a multifunctional biostimulant formulated with brassinosteroids, 1-tricontanol, and vitamin B for corn cultivation. The data showed that this product stimulated the growth of the beneficial microbial population in the soil in such a way that

root growth improved and the photosynthesis rate of the plants increased, improving the agronomic yield of the crop.

2.8. Phospholipids

The Cultiva Corporation [50], a Spanish socio-environmental awareness organization, demonstrated that the application of a product enriched with phospholipids improved the agronomic performance of a cherry crop subjected to a period of drought stress. This product managed to reduce the accumulation of ethylene and the concentration of H_2O_2 in trees grown under drought conditions, which led to an improvement in fruit quality. This same corporation also presented the results obtained with the product Parka® [51], a product based on phospholipids and polysaccharides, which verified how the product prevents damage caused by excess light and/or high temperatures in apple fruits and pear. It seems that this product is capable of dissipating the excess energy captured by the leaves by stimulating the xanthophyll cycle.

2.9. Other Ingredients and Mixture of Active Ingredients

Dr. Ernesto A. Zavala of the Spanish company Atlántica Agrícola demonstrated that the application of the nematode *Pochonia chlamydosporia* [52], in addition to controlling and reducing fungal attacks on crops, could also stimulate plant growth and reduce the flowering time. It seems that the mode of action of this nematode is related to the stimulation of the jasmonic acid pathway.

The UEB company [53] presented its results on the application of 1-(2-methoxyethyl)-3-(1,2,3-thiadiazol-5yl)urea (MTU) to wheat, barley, and rapeseed plants grown under different stresses such as high temperatures, salinity, and drought. It was concluded that this product increased tolerance to these stresses by increasing the concentration of carotenoids and chlorophylls in the leaves while maintaining the photosynthetic machinery in good condition, specifically the PSI–LHC supercomplex.

Omnia [54] presented a new fertilizer (FertiCoat®) composed of marine algae, humates, fulvates, amino acids, and polymers that coat urea granules. Its application was proven to increase the effective use of fertilizers and crop production.

Brandon Science [55] also developed and presented the product BlocSal® containing a biomolecular complex (PSI-475) obtained through the concept of "precision engineering of biostimulants in plants". This complex is capable of increasing the tolerance of tomato plants to salinity by activating genes involved in Na homeostasis while also improving osmotic adjustment and stimulating metabolic activity.

3. Analytical Techniques

Phenotyping. Dr. Iganico Colonna presented AgriThority [56], which is a platform that helps farmers evaluate the effect of biostimulants on their crops. Many of these products were recently formulated, so in many cases their effect in specific climates, soils, and crops is still unknown.

Laser desorption/ionization spectrometry. The formulation of biostimulant products with microorganisms requires three fundamental steps: (1) isolation, (2) identification, and (3) selection of varieties. In the case of identification, Valagro is working with a novel strategy which is based on the integration of matrix-assisted laser desorption/ionization spectrometry with time-of-flight mass detection (MALDI-TOF-MS) and powerful statistical tools that allow the selection of candidate microorganisms for use as biostimulants [57].

Geolocation. Dr. Guilherme Oliveira from Lallemand Plant Care is working with a geostatistical tool [58] to assess the effect of a large-scale fertilizer and biostimulant application at the plot level rather than mini-plot trials. This tool allows for obtaining data from large-scale experiments with an average accuracy of 85%.

4. Future Perspectives

The congress also had numerous presentations which provided suggestions and an analysis of the future steps that must be taken by the biostimulant sector to consolidate and expand the production and sale of agrochemical products.

In this congress, the foundations were laid for the formulation and generation of Biostimulants 2.0 [59], in which specific biostimulation actions are sought to solve specific problems and for which studies on the mode of action and the genetics of plants and their metabolites are needed. For example, new biostimulants are sought that can increase the activity of enzymes such as RubisCo, superoxide dismutase, and catalase.

The term "precision engineering of biostimulants in plants" appeared [60], referring to the specific use of biomolecules that activate the signaling processes that stimulate the physiological mechanisms of adaptation of plants to stress.

It was highlighted that although biostimulants are not essential for plants, their use is fundamental since the combination of fertilization, biostimulation, and production can be aligned with the effective use of conventional fertilizers [61]. Currently, in production systems used to achieve maximum production, a large amount of conventional fertilizers must be applied in such a way that their effective use decreases considerably.

The use of new technologies for the application of biostimulants was recommended [62]. The use of sensors that monitor the size of the plants in real time can regulate the amount of product to be applied to optimize and increase the effectiveness of the treatments.

Emphasis was made on understanding the relationship of the plant with the soil microbiota to maintain soil fertility, reduce the application of conventional fertilizers, and face the different edaphoclimatic scenarios imposed by climate change [63]. In this sense, it was pointed out that studies related to the compounds exuded by plant roots, such as simple and complex sugars, carbohydrates, suberin and cutin, organic acids, enzymes, and chitin, will be of great interest for improving the interaction between plants and microbiota. In fact, one of the works studied the relationship between root exudates that intervene in the solubilization of phosphorus in the soil (organic acids, amino acids, and carbohydrates), and the microbial population and activity in the soil [64].

It will be necessary to create new standardized protocols to validate and give credibility to the effects of biostimulants on crops before the EU. Researchers in Belgium have created a platform thanks to the BioStimTest project [65], in which all kinds of biostimulants are tested on winter wheat, maize, and potato crops. Bioassays are carried out on model plants, and seed germination tests, biomass and root measurements in hydroponic crops, biomass and physiological measurements of greenhouse crops, and plant yield in field measurements are performed. They also evaluate nitrogen use efficiency and drought tolerance.

As with conventional fertilizers, it is necessary to carry out studies on the mechanisms that intervene and regulate the absorption of biostimulants in the leaves when they are applied foliarly [66]. The prerequisite for efficient absorption is to evenly coat the surface, avoid excessive runoff, and to keep the film as long as possible, which allows the "transport" of compounds. The posterior route from the leaf surface to the interior is comprised by two different routes: penetration through the wax and cuticle layers and the stomatal route. While the intercellular spaces in the cuticles have dimensions of a few nm at best, the stomatal pore allows the passage of larger compounds and microorganisms. The driving force for this translocation is the concentration gradient that regulates the diffusion of biostimulants. To improve the absorption of biostimulants, the external concentration should be as high as possible. This is limited, however, when osmotically active substances are applied, which cause water loss and leaf burn. It must also be taken into account that the rate of diffusion decreases with the increase in the size of the compounds that are applied.

The Plant-DiTech researchers proposed changing the methodology used to select drought-tolerant plants [67]. Currently, in these experiments, the plants that survive extreme drought conditions are selected, but in most cases they do not coincide with the most productive plants in conditions of water deficit. In these experiments, the differences

in the rate of transpiration of each plant are in the background, so the levels of drought are not the same for each variety tested. The authors intend to study their physiological responses and see how plants regulate transpiration and stomatal control throughout the experiment, not only when the plant is stressed by the lack of water in the soil.

The current status of the new European legislation that will regulate fertilizer products, no. 2019/1009 [68,69], avoids different standards and regulations for each European country, which are currently limiting the development and expansion of the biostimulant industry.

Dr. Cinzia Margherita, from the University of Torino, presented the ROOT project [70], which intends to study the physiological and molecular responses of plant roots to abiotic stress and how these responses change with the active ingredients used to formulate biostimulant products, such as tannins. Omic sciences such as transcriptomics (RNA-Seq) and metabolomics (LC-MS) are used in this project.

Dr. Eric Lee highlighted the importance of using biorefineries [71] to create products of interest to agriculture from wet milling processes, fermentation, etc. from agro-industrial waste. These biorefineries make possible the production of products rich in polyols, organic acids, etc. that improve stress tolerance, nutrient assimilation, and root growth.

Dr. Guillaume Wegria, from Fyteko, stated that the new formulation of biostimulants will include low molecular weight molecules [72] capable of inducing a cascade of signals that activate multiple bioprocesses related to stress tolerance mechanisms, with these molecules being produced in a sustainable and economic way.

Finally, the importance of developing biostimulants with microorganisms included in EU regulation 2019/1009 [73] was highlighted, for which it is necessary to isolate and characterize soil microorganisms and select those that have a high capacity to solubilize P and K and fix N. These strains could be used to formulate biostimulants in both liquid and solid form.

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