Presence and future of plant phenotyping approaches in biostimulant research and development

Nuria De Diego and Lukáš Spíchal*

Centre of the Region Haná for Biotechnological and Agricultural Research, Czech Advanced Technology and Research Institute (CATRIN), Palacký University Olomouc, Šlechtitelů 27, Olomouc CZ-783 71, Czech Republic

* Correspondence: lukas.spichal@upol.cz

Received 19 November 2021; Editorial decision 16 June 2022; Accepted 20 June 2022

Editor: Michela Janni, National Research Council, Italy

Abstract

Commercial interest in biostimulants as a tool for sustainable green economics and agriculture concepts is on a steep rise, being followed by increasing demand to employ efficient scientific methods to develop new products and understand their mechanisms of action. Biostimulants represent a highly diverse group of agents derived from various natural sources. Regardless of their nutrition content and composition, they are classified by their ability to improve crop performance through enhanced nutrient use efficiency, abiotic stress tolerance, and quality of crops. Numerous reports have described modern, non-invasive sensor-based phenotyping methods in plant research. This review focuses on applying phenotyping approaches in biostimulant research and development, and maps the evolution of interaction of these two intensively growing domains. How phenotyping served to identify new biostimulants, the description of their biological activity, and the mechanism/mode of action are summarized. Special attention is dedicated to the indoor high-throughput methods using model plants suitable for biostimulant screening and developmental pipelines, and high-precision approaches used to determine biostimulant activity. The need for a complex method of testing biostimulants as multicomponent products through integrating other -omic approaches followed by advanced statistical/mathematical tools is emphasized.

Keywords: High-throughput screening, mechanism of action, mode of action, -omics, plant biostimulants, plant breeding, plant phenotyping, sensors.

Introduction

‘Plant biostimulants’ is a hypernym used to describe very different substances such as seaweed extracts, humic and fulvic acids, animal- and vegetal-based protein hydrolysates, and microorganisms such as mycorrhizal fungi and rhizospheric bacteria. The definition of plant biostimulants has been the subject of extensive discussions and evolution to promote the acceptance of biostimulants by future regulations (du Jardin, 2015; Yakhin et al., 2017; du Jardin et al., 2020). The first legal definition was provided in the US Farm Bill (Agriculture Act of 2018; https://www.congress.gov/115/bills/hr2/BILLS-115hr2enr.pdf) describing a plant biostimulant as ‘a substance or microorganism that, when applied to seeds, plants, or the rhizosphere, stimulates natural processes to enhance or benefit nutrient uptake, nutrient efficiency, tolerance to abiotic stress, or crop quality and yield’. This definition is consistent with the definition of EU Fertilising Products Regulation 2019/1009 describing ‘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: nutrient use efficiency, tolerance to abiotic stress, quality traits, or availability of confined nutrients in the soil or rhizosphere (https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32019R1009&from=EN).

During the last decade, the landscape of the biostimulant industry has changed. Large global companies entered this domain previously occupied mainly by small and medium-sized enterprises. A simple internet web search using the term ‘global biostimulants market size’ offers numerous market reports estimating the average growth at a compound annual growth rate (CAGR) of ~12% during the next 5 years to reach more than US$ 5 billion by 2027. This continually increasing commercial interest in the development and use of biostimulant products, driven by economic and socio-political factors, has been followed by entering the regulatory framework to establish clear standards regarding the claims made by biostimulant producers. For example, in Europe, a CE-certified biostimulant can be placed in the EU Single Market if it fulfills the safety requirements of Regulation (EU) 2019/1009. Besides, the producer must demonstrate plant biostimulant effect to justify the claims following the EU-harmonized standards defined by the Committee for European Standardization (CEN). The biostimulant trials are becoming more demanding and necessitate rigorous and professional practices to fulfill the claims.

Along with biostimulant development, companies have started interacting with the scientific domain and investing significant parts of their revenues into their research and development activities. Scientific approaches that help identify efficient sources for developing new products, clearly and rigorously describe their effects, and, importantly, help understand their mechanism of action are wanted. Testing biostimulant effects in field conditions is highly relevant and irreplaceable; however, some of the claims, such as abiotic stress tolerance, can hardly be justified in the same field trial. In this regard, modern plant phenotyping using non-invasive digital technologies can speed up the development and characterization of the new biostimulants using high-throughput and high-precision approaches in controlled and semi-controlled conditions. They allow multi-dimensional testing combining different types of application in a broad range of concentrations on plants subjected to several growth conditions, individually or in combination. This review analyzes the evolution of using such approaches in an intensively growing biostimulant research and development domain.

Plant phenotyping and biostimulants: old terms for modern science

Literally, ‘plant phenotyping’ refers to a quantitative description of the plant’s anatomical, ontogenetical, physiological, and biochemical properties resulting from genotypic differences and the environmental conditions to which a plant has been exposed. The use of this term in the scientific literature has been evolving. To overview this evolution, we searched under the Basic Search tab on the Web of Science ‘plant AND phenotyping’ in all fields and refined the results by categories: Plant Science, Agronomy, Horticulture, Environmental Science, and Agriculture Multidisciplinary. We obtained 3826 results, including 3255 original articles, 396 review articles, and 171 proceeding papers. The term plant phenotyping was first mentioned in Cenis et al. (1992). This study focused on the phenotyping of selected enzymes involved in root-knot nematode symptoms (Fig. 1A). After that, the term ‘phenotyping’ was used more in the sense of its literal definition in plant genetic studies to describe quantitative morphological, biochemical, or biological traits that can be attributed to the marker alleles segregating in a well-defined population as quantitative trait loci (QTL) (Bonierbale et al., 1994; Sripongpangkul et al., 2000). ‘Plant phenotyping’ in the modern sense is a quantitative description of the plant’s anatomical, ontogenetical, physiological, and biochemical traits. The analysis is mainly performed using non-invasive methods to measure plant growth and physiology dynamics over time (Walter et al., 2015). Granier et al. (2006) was the first publication using this concept, and the authors presented PHENOPSIS, an automated platform for reproducible phenotyping of plant responses to soil water deficit. Later on, Walter et al. (2007) published GROWSCREEN, a novel set up using a simple camera to analyze plant growth. The number of articles presenting plant phenotyping as a non-invasive approach to analyzing plant performance, including morphology and physiology, has reached 579 published articles and 17 593 citations in 2020. To identify research communities historically involved primarily in plant phenotyping, we analyzed the contribution by country/region according to the published affiliations. As shown in Fig. 1B, researchers from the USA were the most prolific publishers, appearing in 28.725% of the publications, followed by Germany and France with 14.663% and 11.187%, respectively.

To go further on the impact of ‘plant phenotyping’ in the research community, we analyzed a corpus consisting of the 3826 obtained publications using VOSviewer V. 1.6.17 (Centre for Science and Technology Studies, University of Leiden) as described by Ruwona and Scherm (2022). This software uses the VOS (visualization of similarities) technique to produce a network map in which the distance among the terms (nodes) reflects their similarities as closely as possible. First, the 3826 publications directly downloaded from the Web of Science were integrated into an Excel file (corpus). The corpus was analyzed for the co-occurrence of terms within titles, abstracts, and keywords, adding the condition of 80 as a minimum number of occurrences per term. The result was a map with three clusters (Fig. 1C); a red cluster contained the highest number of items (66), a blue cluster had 57 items, and a green cluster had 44 items. The red items were dominated by terms related to soil or rhizosphere: nutrient use efficiency, tolerance to abiotic stress, quality traits, or availability of confined nutrients in the soil or rhizosphere; a blue cluster was dominated by terms for modern science; a green cluster had 57 items, and a blue cluster had 57 items, and a green cluster had 44 items, and a green cluster had 11 items.
Fig. 1. Results related to ‘plant AND phenotyping’ according to the Web of Science using the categories Plant Science, Agronomy, Horticulture, Environmental Science, and Agriculture Multidisciplinary (n=3826). (A) Temporal trend in annual numbers of publications (bars) and citations (blue line). (B) Top countries based on authorship affiliations. (C) A network visualization showing three clusters corresponding to different research themes within the field based on the analysis of the co-occurrence of terms in the titles, abstracts, and keywords of the obtained publications. Minimum number of occurrences per node=80. (D) Overlay visualization depicting the evolution of research terms over time.
related to ‘plant breeding’, containing terms such as ‘population, gene, line, marker, and QTL’, among others. The blue cluster is led by terms such as ‘image, system, technique, technology, model, application, or sensor’, and, to a lesser extent, some terms related to ‘data’ and ‘data analysis’ such as ‘dataset, estimation, and algorithm’. The prevalence terms in the last items (green) were more related to ‘plant response’, including words such as ‘growth, leaf or root’ and ‘environmental conditions’ such as ‘water, drought’, and the model plant ‘Arabidopsis thaliana’. Next, the evolution of these terms over time was analyzed using overlay visualization (Fig. 1D). Since the term ‘plant phenotyping’ in publications increased from 2016, most terms appeared in publications in the last 3 years. Terms with early average publication dates (blue and violet, Fig. 1D) predominantly belonged to the first cluster (red in Fig. 1C) focused on plant breeding or scientific studies using Arabidopsis as a plant model. In 2017, the publications related to plant phenotyping changed the focus to experiments using ‘systems’ and ‘techniques’ to measure the plant response to different growth conditions. The latest average publication dates included ‘image’ and ‘sensor’ or terms such as ‘dataset’ and ‘deep learning technologies’ including ‘accuracy and prediction’, pointing to the new ‘sensor to knowledge’ trend in plant phenotyping. Although plant phenotyping is considered an important tool to be used in the plant breeding process (reviewed by Pieruschka and Schurr, 2019), surprisingly, our analyses of the scientific literature show that plant phenotyping is not evolving to support this and that the distance between breeders and scientists is increasing over time (Fig. 1C, D). Several barriers to overcome between plant phenotyping and plant breeding, such as limited seed supply in the segregating generation following the cross, effects of interplant competition, effects of soil and spatial heterogeneity, statistical analysis issues, estimation of genetic gain and response to selection, the relevance of plant (epi)genomics, and automation challenges, were in this context named by Fasoula et al. (2020). Recently published papers reporting the use of phenotyping in genome-wide association studies (GWAS) and transcriptome-wide association studies (TWAS) are good indicators that this trend will not be long lasting (Yang et al., 2014; Chawade et al., 2019; Li et al., 2020; Ferguson et al., 2021).

We searched under the Basic Search tab of the Web of Science ‘plant AND biostimulator OR biostimulant’ using the same criteria. We obtained 977 results, including 750 articles, 98 review articles, and 111 proceedings papers. The first use of the term ‘plant biostimulator’ appeared in Sanders et al. (1990) (Fig. 2A). This study focused on the effect of two commercial biostimulants (Enersol from humic acids and Ergostim from folic acid) in carrot emergence and root development. As the main results, the authors showed that the exogenous application of both biostimulants improved seedling emergence and root weight. At this time, the term ‘biostimulator’ or ‘biostimulator’ was used to define various substances, including synthetic molecules such as the phytohormone 6-benzylaminopurine or the fungicide propiconazole (Goatley and Schmidt, 1991). As observed in Fig. 2A, the scientific interest in biostimulants started a bit later than in the case of plant phenotyping, and the number of publications per year (~5) did not increase until 2008. In this year, the publications and citations related to plant biostimulants increased exponentially. In 2020 the terms ‘plant AND biostimulator OR biostimulator’ were recorded in 217 articles and 3969 citations. Of all these publications, 24.463% came from scientists with Italian affiliation, followed by 16.991% and 9.314% from Poland and the USA, respectively (Fig. 2B). It is worth mentioning that over the years, the definition of biostimulator evolved, and recently in both North America and Europe, the definition has been unified and is legally binding (as described in the Introduction).

The 977 publications related to plant biostimulants were also analyzed using VOSviewer. Due to the lower number of publications, the minimum number of occurrences per term was reduced to 30. Again a map formed by three main clusters was obtained: a red cluster contained almost half of the items (49), a blue cluster with 36 items, and a green cluster with 18 items (Fig. 2C). The red items were dominated by ‘product, compound, or plant biostimulator’ and other terms such as ‘stress, tolerance, mechanism, and agriculture’. The second item (green) was formed by terms such as ‘control and experiment’ and many others related to morphological and physiological traits such as ‘parameters, weight, value, dry weight, chlorophyll, or antioxidant capacity’. The last items (blue) contained unrelated terms such as factor, year, cultivar, influence, season, etc. When the overlay visualization was performed, we observed that the average publication dates for these terms appear from 2017 to 2019. The first mentioned terms are mainly ‘product, experiment’ or, in lower numbers, ‘substance, biostimulator, influence, or fertilization’ (violet). Most of the terms have an average publication date in 2018 (blue to green). They include terms related to the biostimulant itself, such as ‘compound, role, action’, and the experimental set up such as ‘control, number, parameters, stress, tolerance’, among others, showing that this year was the most relevant for the evolution of the keywords in plant biostimulator research. The latest terms were ‘mechanism, drought, drought stress, salt, and untreated plants’. Similar to the previous analysis, we could observe a fast evolution in plant biostimulator research, starting with introducing new products and substances mainly for fertilization of the plants through a more exhaustive study of the plant response at morphological and physiological levels to end in the description of the mechanism. Many of these studies are reporting successful use of a biostimulator application in the early stages of a plant’s development (Amooaghaie and Moghym, 2011; Khan et al., 2017; Batool et al., 2020). However, recent studies were focused on the biostimulator effect on plant production under different conditions (Khan et al., 2021; Rashid et al., 2021a, b; Jiménez-Arias et al., 2022). Interestingly, in the VOSviewer analysis, no term strictly related to plant phenotyping appears in any of the three items on the map.
Fig. 2. Results related to ‘plant AND biostimulant OR biostimulator’ according to the Web of Science using the categories Plant Science, Agronomy, Horticulture, Environmental Science, and Agriculture Multidisciplinary (n=977). (A) Temporal trend in annual numbers of publications (bars) and citations (blue line). (B) Top countries based on authorship affiliations. (C) A network visualization showing three clusters corresponding to different research themes within the field based on the analysis of the co-occurrence of terms in the titles, abstracts, and keywords of the obtained publications. Minimum number of occurrences per node=30. (D) Overlay visualization depicting the evolution of research terms over time.
Plant phenotyping and biostimulants: trendy scientific topics without a connection

Plant phenotyping and biostimulants generated interest in the scientific communities simultaneously. However, no interconnections between both terms appear in the VOS analysis. To evaluate the degree of connection between both scientific communities, we performed a new search on the Web of Science using ‘plant AND phenotyping AND biostimulant OR biostimulator’ as keywords. We found only 16 results, comprising 12 original works and four review articles (Fig. 3A, B; Tables 1, 2). More than half of the publications originate from scientists with Italian affiliation, followed by the Czech Republic with 31.25% (Fig. 3C). The first publication integrating both scientific topics appeared in 2012 as an opinion article published in Acta Horticulturae by Summerer et al. (2013) resulting from the I. World Congress on the Use of Biostimulants in Agriculture organized in Strasbourg (France) (Table 1). There, the authors pointed to high-throughput phenotyping as a more efficient approach than traditional bioassays for simultaneous comparison of the inducing potential of chemical substances (i.e. hormones) with molecules of proven biostimulant activity. They also described three sensors as non-invasive methods for uncovering the biostimulators’ effects on plants. During this congress, an additional two studies as proceeding papers were presented in which the terms ‘phenomics AND biostimulators’ (but not ‘phenotyping’) appeared together (Petrozza et al., 2012a, b). The two studies are an excellent example of studying the biostimulant effect on plants (in this case, tomatoes) using the described sensors. However, the first original research integrating the topics ‘plant AND phenotyping AND biostimulant OR biostimulator’ as keywords appeared 3 years later, with the highest number of publications (six) and citations (123) recorded in 2019 and 2020, respectively (Fig. 3B).

The 16 identified publications using ‘plant AND phenotyping AND biostimulant OR biostimulator’ as keywords were analyzed using VOSviewer to understand the actual state of the art of plant phenotyping and biostimulant connection. Due to the low number of works, this time, the minimum number of occurrences was reduced to four, ending up with 17 items divided into two clusters; red (nine) and green (eight) (Fig. 3D). The first item (red) is formed by terms such as ‘plant, treatment, application, use, high throughput phenotyping’ and parameters such as ‘plant growth and chlorophyll fluorescence’ and ‘protein hydrolysate’ as the only type of plant biostimulants. The second item (green) is mainly led by ‘biostimulant’ followed by ‘approach, action, crop, development, mode, work, and plant biostimulant’. In addition, the overlay visualization showed that almost all of these terms appeared in the publications at the same time (2018) (Fig. 3E), whereas the average publication date for the terms ‘treatment, mode, and action’ is 2019. These results showed that the studies focused on plant biostimulants using phenotyping approaches are still in development and far from being connected to the plant phenotyping research communities. However, we believe that these technologies can speed up the selection of new products and help understand their mechanism of action described in the perspective article published by Rouphael et al. (2018) (Table 1).

Another critical point nowadays is the evolution of the biostimulant-based products, so they are more focused on complex substances (i.e. seaweed extracts, humic and fulvic acids, animal- and plant-based protein hydrolysates, or formulations that includes microorganisms such as mycorrhizal fungi and rhizospheric bacteria) than on simple natural molecules (i.e. plant hormones or specific amino acids). Thus, the complexity of the new biostimulants due to their natural origin (i.e. seaweeds), the raw material (i.e. animal- and plant-based protein hydrolysates), and/or the preparation procedure needs an indepth study to understand not only their mode of action but also the stability of the batches and viability of the final products. Plant phenotyping has been identified as a beneficial technology for simultaneously testing different batches, extraction processes, and final products, thanks to the high-throughput screening (HTS) approaches (Rouphael et al., 2018).

Indoor phenotyping for plant biostimulant screening and study of the mechanism of action

This section presents insight into the possible use of phenotyping approaches for HTS in biostimulant development. Further, we provide an overview of how indoor phenotyping systems equipped with different sensors were used to describe the effect of plant biostimulant application on traits of interest, pointing to a potential mechanism of action (Table 2).

High-throughput phenotype-based screening approaches using Arabidopsis as a model plant

The first original work was published in 2017 by Burrell et al. The authors introduced a HTS approach for testing libraries of chemical or natural compounds based on in vitro Arabidopsis growth, in which the root and shoot biomass is quantified using simple pictures performed by red–blue–green (RGB) cameras installed in a microphenotron (cabinet) (Burrell et al., 2017). Arabidopsis is a good plant model for screening processes because of its small size and short life cycle. In a microphenotron, a group of Arabidopsis seeds is sown into phytostrips with eight wells each. Then, 12 phytostrips are installed in a 96-well microtiter plate. The cabinet alet allows the simultaneous growth of 27 microtitre plates, giving a total of 2592 seedlings. Due to the small dimensions of the plates, growth can be recorded only for a maximum of 11 d. One limitation of this approach is the difficulty in homogenizing the number of seeds per well due to the tiny size of the Arabidopsis seeds. This fact reduces the accuracy and increases the experimental error.
Fig. 3. Results related to ‘plant AND phenotyping AND biostimulant’ according to the Web of Science using the categories Plant Science, Agronomy, Horticulture, Environmental Science, and Agriculture Multidisciplinary (n=16). (A) Venn diagram representing the publications related to plant phenotyping and/or plant biostimulants. (B) Temporal trend in annual numbers of publications (bars) and citations (blue line). (C) Top countries based on authorship affiliations. (D) A network visualization showing three clusters corresponding to different research themes within the field based on the analysis of the co-occurrence of terms in the titles, abstracts, and keywords of the obtained publications. Minimum number of occurrences per node=4. (E) Overlay visualization depicting the evolution of research terms over time.
of the method. One solution could be to use an appropriate software routine for counting the seed number to normalize the results, as shown by Ugena et al. (2018). Another option is using HTS approaches based on the rosette growth of very young Arabidopsis seedlings individually placed in 48-well plates (Ugena et al., 2018; Sorrentino et al., 2021a; Hernández et al., 2022). This method is performed in a growth chamber in which an XYZ robotic arm is automatically moved above the plates to take RGB images of single plates (more details in De Diego et al., 2017). The advantage of this methodology is that it can simultaneously analyze 572 plates (counting 27,456 seedlings). This means that this ultra-HTS approach allows the simultaneous testing of many compounds from different natural sources at a wide range of concentrations in Arabidopsis seedlings grown under different growth conditions, including osmotic stress, salt stress, or nutritional stress (De Diego et al., 2017; Bryksová et al., 2020).

Additionally, simple RGB imaging offers the extraction of many traits (e.g., rosette growth and plant greenness) that can be integrated to calculate a unique number called the Plant Biostimulant Characterization (PBC) index for a more straightforward classification of biostimulants as plant growth promoters and/or stress alleviators, or growth inhibitors (Ugena et al., 2018; Sorrentino et al., 2021a). Additionally, Sorrentino et al. (2021a) demonstrate the high reproducibility of this ultra-HTS approach for comparing biostimulants from different raw materials. The characterization of plant biostimulants using ultra-HTS approaches also gives a high number of biological replicates (seedlings per variant), which provides enough material to be used in the complementary analysis (i.e., metabolomics) (Sorrentino et al., 2021a). Additionally, the analysis based on Arabidopsis growth has been successful not only in characterizing single compounds or substances but also in studying the impact of the beneficial microorganisms on plant performance and stress response (Sánchez-López et al., 2016; González-Pérez et al., 2018; Fan et al., 2020). However, the number of works describing the use of HTS methods employing model plants such as Arabidopsis for biostimulant screening are today still minimal. HTS technologies are accessible at those phenotyping installations with controlled conditions, mainly localized at scientific institutions, and represent only a minor part of the existing systems. Their accessibility to the biostimulant industry is thus limited.

Moreover, even if they are equipped with simple RGB sensors, professional automated phenotyping platforms can be too expensive for smaller companies. As a solution, several studies have presented low-cost systems for plant phenotyping (Bagley et al., 2020; Ribes et al., 2020; Wu et al., 2020). However, such systems can serve well for scientific purposes but do not necessarily need to meet the routine and standardized screening campaigns called for in industrial practices. From a future perspective, developing more affordable systems and/or devices allowing high-throughput phenotype-based screening with a simple readout using model plants, suitable to be integrated into the biostimulant industries’ pipelines, are needed.

### Phenotyping approaches to study the mechanism of action of plant biostimulants

Yakhin et al. (2017) clearly described the need to distinguish between determining the ‘mode of action’ and ‘mechanism of action’ in order to understand the function of a complex multicomponent product such as a biostimulant. They suggested that ‘the focus of biostimulant research and validation should be upon proof of efficacy and safety and the determination of a broad mechanism of action, without a requirement for a specific mode of action’ (Yakhin et al., 2017). For that, high-precision phenotyping methods employing multiple sensors can be beneficial using model plants or directly using crops of interest. In general, indoor plant phenotyping presents the advantage of control conditions that can be repeated over experiments (Rouphael et al., 2018). Moreover, the biostimulant effects that can hardly be studied in the field conditions, such as abiotic stress tolerance, can be easily performed there. As shown in Table 2, among the crops of the highest agronomical interest, tomato (Solanum lycopersicum L.) is the favorite species for studying biostimulants using plant phenotyping methods. In tomato plants, biostimulants from different sources [complex biostimulants based on a crude bioextract obtained from microalgae or cyanobacteria (Mutale-joan et al., 2020),

### Table 1. Review articles related to ‘plant AND phenotyping AND biostimulant’ according to the Web of Science using the categories Plant Science, Agronomy, Horticulture, Environmental Science, and Agriculture Multidisciplinary

| Title                                                                 | Journal                                      | Reference          |
|----------------------------------------------------------------------|---------------------------------------------|--------------------|
| High-throughput plant phenotyping: a new and objective method to detect and analyze the biostimulant properties of different products | Acta Horticulturae                           | Summerer et al. (2013) |
| Applications of seaweed extracts in Australian agriculture: past, present and future | Journal of Applied Phycology               | Arioli et al. (2015) |
| High-throughput plant phenotyping for developing novel biostimulants: from lab to field or from field to lab? | Frontiers in Plant Science                  | Rouphael et al. (2018) |
| Algae biostimulants: a critical look at microalgal biostimulants for sustainable agricultural practices | Biotechnology Advances                     | Kapoore et al. (2021) |
| Plant species | Biostimulants Application | Growth conditions | Sensors | Interesting traits | Other -omics | References |
|---------------|--------------------------|-------------------|---------|-------------------|-------------|------------|
| Arabidopsis thaliana L. and Eragrostis tef | Ascophyllum nodosum | Spraying Control conditions | RGB | Root and shoot growth | – | Łangowski et al. (2019) |
| Arabidopsis thaliana L. | Eleven protein hydrolysates | Seed priming Control conditions or salinity | RGB (PlantScreen™ XYZ System, PSI) and FluorCam (PlantScreen™ Compact System, PSI) | Plant growth, fluorescence-related parameters, and PBC index | Untargeted metabolomics | Sorentino et al. (2021a) |
| Capsicum annuum L. | Seaweed extract (CL-SW) or metabolite formula (ICL-NewFo1) | Irrigation Control conditions or drought | Lysimeters Plantarray 3.0 platform (Plant-Ditech) | Control conditions: CL-SW increased transpiration, biomass, and yield Drought: ICL-NewFo1 delayed water loss | – | Dalal et al. 2019 |
| Solanum lycopersicum L. | Five prototypes (Valagro) | Drenching Drought | RGB (Scana- lyzer 3D system; LemnaTec GmbH, Aachen, Germany) | All improved digital biomass and water-use efficiency | Drenching better than foliar application. Increased digital biomass and transpiration | Untargeted metabolomics | Paul et al. (2019b) |
| Solanum lycopersicum L. | Eight protein hydrolysates | Spraying Control conditions | RGB (top and side view) and FluorCam (PlantScreen™ Modular System, PSI) | Two products improved root growth rate and growth performance | Untargeted metabolomics | Paul et al. (2019a) |
| Solanum lycopersicum L. | One protein hydrolysate | Spraying or drenching Control conditions or drought | RGB (top and side-view) and FluorCam (PlantScreen™ Modular System, PSI) | Drenching better than foliar application. Increased digital biomass and transpiration | Untargeted metabolomics | Paul et al. (2021) |
| Solanum lycopersicum L. | Commercial glycine betaine | Spraying Control conditions or Drought | Semi-automated multi-chamber whole-canopy system | Photosynthesis, transpiration, and water-use efficiency | Untargeted metabolomics | Antonucci et al. (2021) |
| Solanum lycopersicum L. | 18 Crude bio-extracts (CBEs) obtained from microalgae and cyanobacteria | Drenching Control conditions | Root and shoot length using a ruler | Root and shoot biomass, N, P, and K uptake | Targeted metabolomics | Mutale-joan et al. (2020) |
eight protein hydrolysates from different plant species (Paul et al., 2019a), a commercial product (Petrozza et al., 2014), or a simple product based on glycine betaine (Antonucci et al., 2021) improved plant growth (Table 2). In another work, the foliar spray of three prototypes prepared from seaweed or plant extracts with selected micronutrients improved the digital mass and the green area of *Zea mays* L., hybrid P0423, Pioneer, and *Glycine max* L. Merr. (Briglia et al., 2019). On the other hand, the application of seaweed-based bios- 
stimulant (CL-SW) or a prepared formulation (ICL-NewFo1) in peppers (*Capsicum annuum* L.) showed contrasting results: CL-SW improved plant transpiration, biomass, and final yield under control conditions, whereas ICL-NewFo1 delayed water loss under drought stress (Dalal et al., 2019). Coated seeds of *Triticum turgidum* L. subsp. *durum* (Desf) Husn. with thyme essential oil or the endophyte *Paraburkholderia phytofirmans* (PsJN) activated different strategies in the plants to deal with the water–nutrient stress (Ben-Jabeur et al., 2021). While the plants primed with thyme essential oil maintained water balance, those treated with PsJN increased their leaf thickness and photosynthesis. The type of application in which a bios- 
stimulant is tested can also influence plant performance (Table 2). Drenching with a particular substance based on protein hydrolysates enhanced tomato growth (measured as digital biomass) and transpiration under control and stress conditions compared with foliar application (Paul et al., 2019b). The bio- 
stimulant sources and type of application thus condition the response of the plants, which at the same time depends on the species and growth conditions.

Contrasting methods of analysis, ranging from manual measurement using a ruler to fully automated approaches using multiple sensors, can be found in the literature describing the analysis of the effect of biostimulant application on plants (Paul et al., 2019a; Mutale-joan et al., 2020) (Table 2). However, nowadays, the most preferred plant phenotyping methods are the non-destructive ones that permit the kinetic analysis of the plant’s performance (Humplík et al., 2015); they are much faster and offer simultaneous analysis of a much higher number of individuals. Phenotyping methods based on RBG imaging are the most used to characterize the effects of plant biostimulants, appearing in seven of the 12 original articles reported in Table 2. These have been successfully used to evaluate the biostimu- 

tant effect in plants from very early developmental stages to production (Ugena et al., 2018; Łangowski et al., 2019). However, for a further understanding of the physiological effects, the combination of RGB with FluorCam for measuring

| Plant species | Biostimulants | Application | Growth conditions | Sensors | Interesting traits | Other -omics | References |
|---------------|---------------|-------------|-------------------|---------|-------------------|--------------|-----------|
| *Triticum turgidum* L. subsp. *durum* (Desf) Husn. | Thyme essential oil or *Paraburkholderia phytofirmans* (PsJN) | Seed coating | Control conditions | MultiSpeQ PhotosynQ platform (http://www.photosynq.org) | Control conditions: PsJN increased biomass, leaf thickness, and photosynthesis. Water–nutrient stress: different strategies; thyme essential oil maintained water balance and PsJN leaf thickness and photosynthesis. | Genomics | Ben-Jabeur et al. (2021) |
| *Zea mays* L. cv. Ronaldinho | Plant growth-promoting rhizobacterium [*Bacillus licheniformis* (FMCH001)] | Seed coating | Control conditions or drought | Soil water content and crop coverage. Conveyor system (ProInvent A/S, Hørsholm, Denmark) | Root and shoot dry weight, WUE, and catalase activity | Genomics | Akhtar et al. (2020) |
| *Zea mays* L., hybrid P0423, Pioneer or *Glycine max* L. Merr. | Three prototypes | Spraying | Control conditions | RGB and FluorCam (Scanalyzer 3D system, LemnaTec GmbH) | All improved digital biomass and green area | Genomics | Briglia et al. (2019) |
| – | Lactic acid bacteria and rhizobacteria | Growth medium | Near-infrared (NIR) and UV-visible-NIR (UV-Vis-NIR) spectroscopy | Selection of the interesting bacteria | | | Treguier et al. (2021) |
the fluorescence-related parameters (light phase of plant photosynthesis) permits a deeper analysis of the response of the plants treated with plant biostimulants (Briglia et al., 2019; Sorrentino et al., 2021a). Using lysimeters can be very helpful for water deficit studies, directly informing on the plants’ water loss (Dalal et al., 2019) (Table 2). Alternatively, infrared gas exchange equipment connected to a multichamber whole-canopy system was presented to be used to measure the biostimulant effect on the photosynthetic-related parameters (dark phase) and the plant water use efficiency in tomatoes (Antonucci et al., 2021). More sophisticated sensors (hyperspectral cameras) have also been used for characterizing biostimulants based on beneficial microorganisms. For example, these were used to identify the most efficient Trichoderma spp. strains able to constrain Rhizoctonia solani Kuhn, Sclerotinia sclerotiorum (Lib.) de Bary, and Sclerotium rolfsii Sacc. growth in vitro and to study the effect of these microbes on plants in vivo using baby lettuce as a host (Manganiello et al., 2021). Near-infrared (NIR) and UV-visible-NIR (UV-Vis-NIR) spectroscopy was also used for selecting lactic acid bacteria and rhizobacteria in vitro (Treguier et al., 2021).

Understanding of biostimulant mechanism/mode of action through the integration of other -omics and advanced statistical tools

Well-determined selection of sensor(s) or their combination for the phenotyping experiment can offer the appropriate tool for characterizing the biostimulant’s mechanism(s) of action. Even if biostimulants are mostly complex multicomponent products, we believe that advanced plant phenotyping combined with various -omics approaches followed by data analysis using advanced statistical tools can be used to unravel the potential biostimulant mode of action. Untargeted metabolomics was shown to be a valuable technique to be combined with phenomics for characterizing the potential mode of action of different biostimulants applied to Arabidopsis (Sorrentino et al., 2021a), tomato (Paul et al., 2019a, b; Antonucci et al., 2021; Sorrentino et al., 2021b) or lettuce (Sorrentino et al., 2021b). The challenging part of combining both -omics is the accumulation of a considerable amount of data, requiring suitable tools for analyzing the big datasets. The use of multivariate statistical approaches (frequently employed in metabolomics) allows the classification of the scores (variants) and uncovers the main metabolic differences between untreated and treated plants. Sorrentino et al. (2021b) compared the effect of the same biostimulants in two crop species (tomato and lettuce) and discovered different species-specific responses in control and stress conditions. In this case, the machine learning algorithm defined as random forest classification pointed in tomato to traits related to chlorophyll fluorescence parameters in combination with specific antistress metabolites that benefit the electron transport chain, such as 4-hydroxycoumarin and vitamin K1 (phylloquinone). In lettuce, biomass-related parameters and water use efficiency were the most relevant traits, with a better response connected mainly to plant hormone regulation, especially auxins (Sorrentino et al., 2021b).

Similarly, the effect of three biostimulants in Z. mays L., hybrid P0423, Pioneer, and G. max L. Merr. was studied using a combination of phenotyping with next-generation sequencing (NGS), revealing activation of different species-specific responses (Briglia et al., 2019). In maize, genes involved in hormone (cytokinin and auxin) metabolism/catabolism, maltose biosynthesis, sugar transport, and phloem loading were up-regulated. In contrast, genes involved in nitrogen metabolism, metal ion transport (mainly zinc and iron), sulfate biosynthesis, sugar transport, and phloem loading were up-regulated. In maize, genes involved in nitrogen metabolism, metal ion transport (mainly zinc and iron), sulfate biosynthesis, sugar transport, and phloem loading were up-regulated. In maize, genes involved in nitrogen metabolism, metal ion transport (mainly zinc and iron), sulfate biosynthesis, sugar transport, and phloem loading were up-regulated.
Mathematical tools can also represent essential improvements in the HTS campaigns to identify new biostimulants. A good example is the work by Treguier et al. (2021), where artificial neural network models were presented as tools to correctly identify the genus (species) of 70% (63%) of the lactic acid bacteria and 67% of the rhizobacteria on an independent prediction set of unknown bacterial strains. The integration of plant phenotyping with other -omics can provide additional value to the experimental set ups offering the next step for a deeper understanding of the mode of action of plant biostimulants. Advanced mathematical tools can further contribute to identifying new traits, markers, or metabolic pathways beneficial for the discovery, evaluation, and development of new biostimulants.

Conclusions
Plant phenotyping and biostimulants represent dynamically evolving domains in research and development of the last decade. Interestingly, they show the opposite supply–demand interaction of science and industry. Plant phenotyping technologies appeared on demand from scientists to satisfy their needs for more efficient ways to describe plant phenotypes. On the other hand, industrial producers of biostimulants recognized a need to interact with the scientific domain and searched for new technologies to improve their research and development. Our analysis of the scientific literature showed the evolution of the trends in both fields with recognizable recent and future ‘meeting points’. (i) Plant phenotyping: plant breeding (QTL) → new non-invasive systems to phenotype plants data analysis and deep learning. (ii) Biostimulants: testing products in simple assays → new parameters studied under controlled conditions or stress → mechanism of action in plants under salt and drought conditions. (iii) Plant phenotyping and biostimulants: new approaches for testing crop development induced by biostimulants → type of treatment and their mode of action.

Practically, plant phenotyping technologies seem to become essential for the biostimulant industry. They can significantly contribute to: (i) basic research of biostimulants, and characterization of their mechanism/mode of action; (ii) screening campaigns for the identification of new biostimulants from new raw materials and beneficial microorganisms; (iii) developmental pipelines for analyses of batches and testing of a new method of preparation; (iv) production pipelines for shelf-life analysis and quality control; and (v) justification of the claims requested by certification authorities.

On the contrary, testing biostimulants as complex multicomponent products can stimulate the need to develop new complex phenotyping approaches and methodologies integrating different -omics and robust data analysis tools such as multivariate statistical approaches and artificial intelligence. Multidimensional biostimulant testing combining different application types (seed, foliar, and soil) in broad concentration ranges in multiple environmental conditions requires a high-throughput capacity for testing hardware and analysis software. Higher testing capacities of phenotyping facilities and/or development of affordable devices will be needed for high-throughput phenotype-based screening and data analysis ready for integration into the biostimulant R&D industries’ pipelines. Moreover, we believe that plant phenotyping should reconnect to plant breeding. By doing so, it will open a new perspective of biostimulant testing, offering the possibility of developing targeted biostimulants with a specific activity in crops grown in conditions for which they were primarily selected by breeding programs. An ideal interconnection among the plant phenotyping scientific community, biostimulant industry, and plant breeders is presented in Fig. 4. Ultra-HTS is routinely used to test new technologies related to the final products’ production, batches, stability, and shelf-life. Additionally, the new products are tested in model plants under different growth conditions and, at the same time, compared with well-established products with confirmed activity for faster selection. Once selected, the new products are tested in different crops/varieties/genotypes, preferably suggested by breeders (depending on the interest) under different growth conditions using HTS approaches (early developmental stages). For further information, the studies can be combined with other -omics (e.g. genomics and metabolomics) to better understand the mechanism of action. Finally, the results are evaluated using an integrative plant phenotyping approach indoors and/or outdoors in the plant species of interest. All data must be analyzed using advanced statistical/mathematical tools (machine learning/artificial intelligence) for identifying relevant markers (phenotyping traits) to simplify the pipeline for the selection of new interesting ‘genotypes’ and ‘biostimulants’ together. Hence, genotypes showing a positive response to certain biostimulants can also be selected.

Author contributions
NDD and LS: conceptualization and writing; NDD: bibliographic research and data analysis.

Conflict of interest
No conflict of interest declared.

Funding
This work was supported by the ERDF project ‘Plants as a tool for sustainable global development’ (no. CZ.02.1.01/0.0/0.0/16_019/0000827) from the Ministry of Education, Youth and Sports of the Czech Republic.

Data availability
The complete list of the source publications used for VOSviewer analyses presented in this publication are available from the corresponding author, Lukáš Spíchal, upon request.
References

Akhtar SS, Amby DB, Hegelund JN, Fimognari L, Großkinsky DK, Westergaard JC, Müller R, Moelbak L, Liu F, Roitsch T. 2020. Bacillus licheniformis FMC6001 increases water use efficiency via growth stimulation in both normal and drought conditions. Frontiers in Plant Science 11, 297.

Amooaghaie R, Moghym S. 2011. Effect of polyamines on thermotolerance and membrane stability of soybean seedling. Journal of Biotechnology 10, 9673–9679.

Antonucci G, Croci M, Miras-Moreno B, Fracasso A, Amaducci S. 2021. Integration of gas exchange with metabolomics: high-throughput phenotyping methods for screening biostimulant-elicited beneficial responses to short-term water deficit. Frontiers in Plant Science 12, 678925.

Arioli T, Matthner SW, Winberg PC. 2015. Applications of seaweed extracts in Australian agriculture: past, present and future. Journal of Applied Phycology 27, 2007–2015.

Bagley SA, Atkinson JA, Hunt H, Wilson MH, Pridmore TP, Wells DM. 2020. Low-cost automated vectors and modular environmental sensors for plant phenotyping. Sensors 20, 3319.

Batool S, Khan S, Basra SMA. 2020. Foliar application of moringa leaf extract improves the growth of moringa seedlings in winter. South African Journal of Botany 129, 347–353.

Ben-Jabeur M, Gracia-Romero A, López-Cristofanini C, Vicente R, Kthiri Z, Kefaurov SC, López-Carbonell M, Serret MD, Araus JL, Hamada W. 2021. The promising MultiSensQ device for tracing the effect of seed coating with biostimulants on growth promotion, photosynthetic state and water–nutrient stress tolerance in durum wheat. Euro-Mediterranean Journal for Environmental Integration 6, 8.

Bonierbale MW, Plaisted RL, Pineda O, Tanksley SD. 1994. QTL analysis of trichome-mediated insect resistance in potato. Theoretical and Applied Genetics 87, 973–987.

Briglia N, Petrozza A, Hoebberichts FA, Verhoef N, Povero G. 2019. Investigating the impact of biostimulants on the row crops corn and soybean using high-efficiency phenotyping and next generation sequencing. Agronomy 9, 761.

Brysková M, Hybenová A, Hernáničká AE, Novák O, Pěčínk A, Spichal L, De Diego N, Doležel K. 2020. Homopriming to mitigate abiotic stress effects: a case study of N9-substituted cytokinin derivatives with a fluorinated carbohydrate moiety. Frontlines in Plant Science 11, 506225.

Burrell T, Fozard S, Holroyd GH, French AP, Pound MP, Bigley CJ, Čík A, Spíchal L, Číkář J, Opperman C, Triantaphyllou A. 1992. Cytogenetic, enzymatic, and precision agriculture. Agronomy 258.

Chawade A, Van Ham J, Blomquist H, Bagge O, Alexandersson E, Ortiz R. 2019. High-throughput field-phenotyping tools for plant breeding and precision agriculture. Agronomy 9, 258.

Dalal A, Bourstein R, Haish N, Shenhar I, Wallach R, Moshelion M. 2019. Dynamic physiological phenotyping of drought-stressed pepper plants treated with “productivity-enhancing” and “survivability-enhancing” biostimulants. Frontiers in Plant Science 10, 905.

Danzí D, Briglia N, Petrozza A, Summerer S, Povero G, Stivaletta A, Cellini F, Pignone D, De Paola D, Janin M. 2019. Can high throughput phenotyping help food security in the Mediterranean area? Frontiers in Plant Science 10, 15.

De Diego N, Fürst T, Humphlik JF, Ugena L, Podlešáková K, Spichal L. 2017. An automated method for high-throughput screening of arabidopsis rosette growth in multi-well plates and its validation in stress conditions. Frontiers in Plant Science 8, 1702.

du Jardin P. 2015. Plant biostimulants: definition, concept, main categories and regulation. Scientia Horticulturae 186, 3–14.

du Jardin P, Xu L, Geelen D. 2020. Agricultural functions and action mechanisms of plant biostimulants (PBs): an introduction. In: Geelen D, Xu L, eds. The chemical biology of plant biostimulators. Chichester, UK: Wiley, 1–30.

Fan D, Subramanian S, Smith DL. 2020. Plant endophytes promote growth and alleviate salt stress in Arabidopsis thaliana. Scientific Reports 10, 12740.

Fasoula DA, Ioannides IM, Omiroiu M. 2020. Phenotyping and plant breeding: overcoming the barriers. Frontiers in Plant Science 10, 1713.

Ferguson JN, Fernandes SB, Monier B, et al. 2021. Machine learning-enabled phenotyping for GWAS and TWAS of UWE traits in 869 field-grown sorghum accessions. Plant Physiology 187, 1481–1500.

Goatley JM, Schmidt RE. 1991. Biostimulator enhancement of Kentucky bluegrass sod. HortScience 26, 254–255.

González-Pérez E, Ortega-Amaro MA, Salazar-Badillo FB, Bautista E, Doutlerunge D, Jiménez-Bremont JF. 2016. The Arabidopsis–Trichoderma interaction reveals that the fungal growth medium is an important factor in plant growth induction. Scientific Reports 8, 16427.

Granier C, Aguirrezabal L, Chenu K, et al. 2006. PHENOPSIS, an automated platform for reproducible phenotyping of plant responses to soil water deficit in Arabidopsis thaliana permitted the identification of an accession with low sensitivity to soil water deficit. New Phytologist 169, 623–635.

Hernández AE, Aucique-Perez CE, Čávar Željković S, Štefelová N, Salcedo Sarmiento S, Spichal L, De Diego N. 2022. Priming with small molecule-based biostimulants to improve abiotic stress tolerance in Arabidopsis thaliana. Plants 11, 1287.

Humphlik JF, Lazar D, Husišková A, Spichal L. 2015. Automated phenotyping of plant shoots using imaging methods for analysis of plant stress responses—a review. Plant Methods 11, 29.

Jiménez-Arias D, Hernández AE, Morales-Sierra S, García-García AL, García-Machado FJ, Luís JC, Borges AA. 2022. Applying biostimulants to combat water deficit in crop plants: research and debate. Agronomy 12, 571.

Kapoor RV, Wood EE, Llewellyn CA. 2011. Algae biostimulants: a critical look at microalgal biostimulants for sustainable agricultural practices. Biotechnology Advances 49, 107754.

Khan S, Basit A, Hafeez MB, et al. 2021. Moringa leaf extract improves biochemical attributes, yield and grain quality of rice (Oryza sativa L.) under drought stress. PLoS One 16, e0254452.

Khan S, Basra SMA, Afzal I, Nawaz M, Rehman HU. 2017. Growth promoting potential of fresh and stored Moringa oleifera leaf extracts in improving seedling vigor, growth and productivity of wheat crop. Environmental Science and Pollution Research 24, 27601–27612.

Langowski L, Goñi O, Quille P, Stephenson P, Carmody N, Feeney E, Barton D, Östergaard L, O’Connell S. 2019. A plant biostimulator from the seaweed Ascophyllum nodosum (Sealikit) reduces podshatter and yield loss in oilseed rape through modulation of IND expression. Scientific Reports 9, 16644.

Li B, Chen L, Sun W, et al. 2020. Phenomics-based GWAS analysis reveals the genetic architecture for drought resistance in cotton. Plant Biotechnology Journal 18, 2533–2544.

Manganiello G, Nicastro N, Caputo M, Zaccardelli M, Cardi T, Pane E, Douterlungne D, Jiménez-Bremont JF, González-Pérez E, Luis JC, Borges AA, 2022. Applying biostimulants to combat water deficit in crop plants: research and debate. Agronomy 12, 571.

Moreda JF, García-Machado FJ, Luís JC, Borges AA. 2022. Applying biostimulants to combat water deficit in crop plants: research and debate. Agronomy 12, 571.

Paul K, Sorrentino M, Lucini L, et al. 2019a. Understanding the bios- stimulant action of a combined phenotypic and metabolomic approach for elucidating the biostimulant action of a
plant-derived protein hydrolysate on tomato grown under limited water availability. Frontiers in Plant Science 10. 493.

Petrozza A, Santaniello A, Summerer S, Di Tommaso G, Di Tommaso D, Paparelli E, Piaggesi A, Perata P, Cellini F. 2014. Physiological responses to Megafol® treatments in tomato plants under drought stress: a phenomic and molecular approach. Scientia Horticulturae 174, 185–192.

Petrozza A, Summerer S, Tommaso GD, Tommaso DD, Piaggesi A. 2012a. Evaluation of the effect of RADIFARM® treatment on the morpho-physiological characteristics of root systems via image analysis. Acta Horticulturae 1009, 149–154.

Petrozza A, Summerer S, Tommaso GD, Tommaso DD, Piaggesi A. 2012b. An evaluation of tomato plant root development and morpho-physiological response treated with VIVA® by image analysis. Acta Horticulturae 1009, 155–160.

Pieruschka R, Schurr U. 2019. Plant phenotyping: past, present, and future. Plant Phenomics 2019, 7507131.

Rashid N, Khan S, Wahid A, et al. 2021a. Exogenous application of moringa leaf extract improves growth, biochemical attributes, and productivity of late-sown quinoa. PLoS One 16, e0259214.

Rashid N, Khan S, Wahid A, et al. 2021b. Exogenous application of bio-stimulants and synthetic growth promoters improved the productivity and grain quality of quinoa linked with enhanced photosynthetic pigments and metabolomics. Agronomy 11, 2302.

Ribes M, Russias G, Tregot A, Fournier A. 2020. Towards low-cost hyperspectral single-pixel imaging for plant phenotyping. Sensors 20. 1132.

Rouphael Y, Spichal L, Panzarová K, Casa R, Colla G. 2018. High-throughput plant phenotyping for developing novel biostimulants: from lab to field or from field to lab? Frontiers in Plant Science 9, 1197.

Ruwona J, Scherm H. 2022. Sensing and imaging of plant disease through the lens of science mapping. Tropical Plant Pathology 47, 74–84.

Sánchez-López AM, Baslam M, De Diego N, et al. 2016. Volatile compounds emitted by diverse phytopathogenic microorganisms promote plant growth and flowering through cytokinin action. Plant, Cell & Environment 39, 2592–2608.

Sanders DC, Ricotta JA, Hodges L. 1990. Improvement of carrot stands with plant biostimulants and fluid drilling. HortScience 25, 181–183.

Sorrentino M, De Diego N, Ugena L, Spichal L, Lucini L, Miras-Moreno B, Zhang L, Rouphael Y, Colla G, Panzarová K. 2021a. Seed priming with protein hydrolysates improves Arabidopsis growth and stress tolerance to abiotic stresses. Frontiers in Plant Science 12. 626301.

Sorrentino M, Panzarová K, Spyroglou I, Spichal L, Buffagni V, Ganugi P, Rouphael Y, Colla G, Lucini L, De Diego N. 2021b. Integration of phenomics and metabolomics datasets reveals different mode of action of biostimulants based on protein hydrolysates in lettuce and tomato under salinity. Frontiers in Plant Science 12, 808711.

Sripongpangkul K, Posa GBT, Senadhira DW, Brar D, Huang N, Khush GS, Li ZK. 2000. Genes/QTLs affecting flood tolerance in rice. Theoretical and Applied Genetics 101. 1074–1081.

Summerer S, Petrozza A, Cellini F. 2013. High throughput plant phenotyping: a new and objective method to detect and analyse the biostimulant properties of different products. Acta Horticulturae 1009, 143–148.

Treguier S, Couderc C, Audonnet M, Mzali L, Tormo H, Daveran-Mingot M-L, Ferhout H, Kleiber D, Levasseur-Garcia C. 2021. Identification of lactic acid bacteria and rhizobacteria by ultraviolet–visible–near infrared spectroscopy and multivariate classification. Journal of Near Infrared Spectroscopy 29, 096703352110359.

Ugena L, Hýlová A, Podlešáková K, Humplík JF, Doležal K, De Diego N, Spichal L. 2018. Characterization of biostimulant mode of action using novel multi-trait high-throughput screening of arabidopsis germination and rosette growth. Frontiers in Plant Science 9, 1327.

Walter A, Liebisch F, Hund A. 2015. Plant phenotyping: from bean weighing to image analysis. Plant Methods 11, 14.

Walter A, Scharr H, Gilmer F, et al. 2007. Dynamics of seedling growth acclimation towards altered light conditions can be quantified via GROWSCREEN: a setup and procedure designed for rapid optical phenotyping of different plant species. New Phytologist 174, 447–455.

Wu S, Wen W, Wang Y, Fan J, Wang C, Gou W, Guo X. 2020. MVS-Pheno: a portable and low-cost phenotyping platform for maize shoots using multiview stereo 3D reconstruction. Plant Phenomics 2020, 1848437.

Yakhin OI, Lubyanov AA, Yakhin IA, Brown PH. 2017. Biostimulants in plant science: a global perspective. Frontiers in Plant Science 7, 2049.

Yang W, Guo Z, Huang C, et al. 2014. Combining high-throughput phenotyping and genome-wide association studies to reveal natural genetic variation in rice. Nature Communications 5, 5087.