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Characterization of biostimulants using novel high-throughput screening approaches in plants under different stress conditions.

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Contents

Abstract

Plant response to stress is a highly dynamic and complex process dependent on the severity and duration of the stress, the fitness and preparedness of the plant itself and its developmental stage. Breeders worldwide have therefore focused on quantitative analyses of plant traits in order to accelerate the development of appropriate strategies for improving crops which are adaptable to resource-limited environments. Soil salinity is an important environmental factor that reduces plant germination and early seedling establishment and results in decreased crop productivity on a global scale.

The application of biostimulants represents one of the most innovative and promising strategies for minimizing stress impact, including salinity. The origin of biostimulants is diverse, and ranges from single compounds to complex matrices with different groups of bioactive components that have only been partly characterized. Irrespective of their complexity, biostimulants encompass different groups of plant signaling compounds such as plant hormones, amino acids, and polyamines among others. The exogenous application of these signaling molecules has been reported to ameliorate the adverse effect of stress through sophisticated crosstalk leading to the activation of conserved pathways. Their use also contributes to more sustainable and environmentally friendly agricultural practice, and offers an alternative to synthetic protectants.

Plant phenotyping platforms have become an important tool in plant biology and agriculture. They provide new possibilities for automated, fast scoring of several plant growth and development traits, followed over time using non-invasive sensors. These approaches allow simultaneous testing of a large number of potentially bioactive compounds in a wide range of concentrations and / or genotypes, under various growth conditions as well providing information about the developmental and physiological status of the treated plants and, analyzing traits like the scoring of seedling emergence. Altogether, we consider that the new protocols based on high-throughput screening (HTS) could accelerate identification of the mode of action of known biostimulants and the characterization of new ones.

Souhrn

Reakce rostlin na stres je vysoce dynamický a složitý proces závislý na síle a trvání stresu, kondici a připravenosti samotné rostliny a na její vývojové fázi. Šlechtitelé po celém světě se proto zaměřili na kvantitativní analýzy vlastností rostlin s cílem urychlit vývoj vhodných strategií pro zlepšení plodin, které jsou přizpůsobivé prostředí s omezenými zdroji. Salinita půdy je důležitým faktorem životního prostředí, který snižuje klíčení rostlin, ranný vývoj semenáčků a má za následek snížení produktivity plodin v globálním měřítku.

Aplikace biostimulantů představuje jednu z nejinovativnějších a nejslibnějších strategií pro minimalizaci stresu, včetně solného. Původ biostimulantů je různorodý a pohybuje se v rozmezí od jednotlivých sloučenin až po komplexní matrice s různými skupinami bioaktivních složek, které byly pouze částečně charakterizovány. Bez ohledu na jejich složitost zahrnují biostimulanty různé skupiny rostlinných signálních molekul jako jsou rostlinné hormony, aminokyseliny a polyaminy. Bylo popsáno, že exogenní aplikace těchto signálních molekul zmírňuje nepříznivý účinek stresu prostřednictvím sofistikované interakce vedoucí k aktivaci konzervovaných drah. Jejich použití také přispívá k udržitelnější zemědělské činnosti šetrné k životnímu prostředí a nabízí alternativu k syntetickým ochranným látkám.

Platformy pro fenotypizaci rostlin se staly důležitým nástrojem v rostlinné biologii a zemědělství. Poskytují nové možnosti automatizovaného a rychlého vyhodnocování různých růstových a vývojových znaků rostlin za využití neinvazivních senzorů. Tyto přístupy umožňují současné testování velkého počtu potenciálně biologicky aktivních sloučenin v širokém koncentračním rozmezí a / nebo genotypů, za různých růstových podmínek, a také poskytují informace o vývojovém a fyziologickém stavu ošetřených rostlin skrze analýzu znaků, např. při vzcházení. Domníváme se, že nové protokoly založené na vysokokapacitním screeningu (HTS) by mohly urychlit identifikaci toho, jak působí známé biostimulanty a pomoci k charakterizaci nových látek.

I. Introduction

Climate change is a problem of the highest priority today, which influences agricultural production worldwide. Climate variability and extreme climate conditions are affecting agricultural production. For these reasons, improved crop production has become a research priority in the past decades [1].

Plants have to endure periods under unfavorable situations throughout their life cycle. In order to survive, plants have developed sophisticated defense mechanisms that act as diverse responses to all these stimuli [2]. According to the factors that produce the stress, these may be "biotic stress" or "abiotic stress", which are the main restrictive factor in agricultural productivity [3,4]. Abiotic stresses are caused by non-living factors, such as drought, changes in temperature and, salinity, among others, that affect the plant's growth, reproduction and life [5,6].

Salinity is one of the major abiotic factors that affect plant growth and productivity [7–9]. High concentration of salt in soil affects plant growth and nutrient uptake in plants inducing some nutrient deficiencies. Consequently, breeders worldwide use different strategies in order to accelerate the development of appropriate methodologies for improving crop production and alleviate the stress conditions.

The most important strategies of crop improvements against stresses include several agronomical, physiological and molecular approaches such as classical breeding programs, molecular breeding, genetic engineering, and/or environmental friendly practices, such as the use of biostimulants, among others [10].

Biostimulants have been gaining interest because their application activates several physiological processes in plants to stimulate growth, improve plant tolerance to environmental disturbances, alleviate stress-induced limitations and to increase yield [11]. One of the definitions formally established was that "plant biostimulants contain substance(s) and/or micro-organisms whose function when applied to plants or the rhizosphere is to stimulate natural processes to enhance/benefit nutrient uptake, nutrient efficiency, tolerance to abiotic stress, and crop quality" [12].

In 2015, it was provided a broad classification into seven groups of compounds reported as biostimulants [13,14]. The majority of biostimulants have an undefined composition made by complex mixtures of compounds derived from a biological process or extracted from biological materials. The interaction of these complex formulations is essential for the performance of the biostimulants as their properties cannot be elucidated a priori by knowing the activity of the individual components. For a large number, no specified mode of action or a mechanism of action has been identified. Therefore, there is a real need to ensure that all products on the market have clear benefits to crop productivity.

High-throughput phenotyping (HTP) has become an important tool in agriculture and contributes significantly to plant breeding and management approaches [15]. They offer the opportunity to combine various automated, simultaneous and non-destructive online methods monitoring multiple morpho-physiological plant traits. In addition, they provide a complex picture of the plant growth and vigour in one run, and time-course measurements during the plant's life-span, showing the progression of growth, reducing cost, labor and time-consuming for sampling and for analyses by improved data integration and remote sensing [16,17]. The broad spectrum of plant traits can be described by integrative phenotyping in multi-sensoric phenotyping platforms including imaging sensors for visible imaging (RGB imaging) and/or 3D imaging, imaging spectroscopy (hyperspectral imaging), thermal infrared imaging, and chlorophyll fluorescence [17–19].

The use of high- throughput phenotyping platforms were recently proposed in order to characterize the biostimulant mode of action and as an efficient tool for finding new bioactive substances [16,20]. Furthermore, the combination of advanced algorithms and statistical data analysis contributes in the success identification of the morpho-physiological markers given by phase or type of biostimulant application or stress response [16,21,22]. Therefore, the use of HTS platforms allows the evaluation of the biostimulant effects in a broad concentration range under different growth conditions or even in combination in a single run and in different plant species [22,23]. Altogether, non-invasive image analysis-based methods have allowed us to classify the effect of a compound application on plants under control or different stress conditions, pointing to this technology as a key for a faster and more efficient characterization of biostimulants.

II. Aims of the Ph.D. thesis

Plant response to stress is a highly dynamic and complex process dependent on the severity and duration of the stress. The application of biostimulants represents one of the most innovative and promising strategies for minimizing stress impact. However, there are obstacles to determining their mode of action. For this reason, the development of efficient, affordable and high-throughput agronomic techniques for identifying and validating the legitimacy of a product on the market of biostimulants is a priority.

The main objectives elaborated and discussed in this doctoral thesis are the following:

- Compilation of a literature review related to the topic of the doctoral thesis, specifically plant stress, biostimulants and high-throughput screening approaches.
- In depth study bringing together information on plants exposed to stress conditions, and discussion of the possible crosstalks among different groups of signaling molecules.
- Development of a highly reproducible *in vitro* HTS bioassay using *Arabidopsis thaliana* as a model plant to be used for selecting phenotypes, growth conditions and/or compounds that can confer stress tolerance.
- Development of a novel multi-trait high-throughput screening (MTHTS) of *Arabidopsis* for the identification of new biostimulants and their modes of action under different salt stress concentrations.
- Characterization of the seedling emergence using high-throughput screening assays in real crops such as maize (*Zea Mays* L.) under salt stress using the indoor phenotyping method and validation of the assay and characterization of the specific mode of action of the biostimulants.

III. Materials and Methods

Plant material and growth conditions.

Optimization and validation of two phenotyping protocols were established in *Arabidopsis thaliana* (accession Col-0) and maize (*Zea mays* L.) hybrid Koblens (KWS Osiva s.r.o., Czech Republic) under control and salt stress conditions. *Arabidopsis* seeds were surface-sterilized with 70% of EtOH and 0.01% Triton-X, sown on square plates (12 cm x 12 cm) containing 0.5x Murashige-Skoog (MS) medium (Murashige and Skoog, 1962) (pH 5.7) supplemented with a gelling agent 0.6% Phytagel (Sigma–Aldrich, Germany) and maintained for 3 days at 4 ºC in the dark. After cold stratification, plates were transferred into a growth-chamber (CMP6010, Conviron Adaptis) with controlled conditions (22ºC, 16/8 h light/dark cycle, a photon irradiance of 120 µmol photons of PAR m^{-2} s^{-1}), and placed in a vertical position. Three days after germination, seedlings of similar size (one seedling per well) were transferred under sterile conditions into the multi-well plates;12-, 24- and 48-well plates (Jetbiofil, Guangzhou, China) with 2.7 mL, 1.3 mL or 0.850 mL of 1x MS medium per well, respectively (pH 5.7; supplemented with 0.6% Phytagel). Plates were sealed with perforated transparent foil allowing gas and water exchange.

To standardized the normal conditions of the protocol, different concentrations of MS (1x, 0.5x, or 0.25x) and sucrose (0, 0.1, or 1%) (pH 5.7; containing 0.6% Phytagel) were used. In salt-stress experiment, 12- and 24-well plates were used filled with 1x MS medium (pH 5.7; containing 0.6% Phytagel) with the addition of NaCl to achieve specific salinities (50, 75, 100, or 150 mM NaCl). To deal with interacting growth conditions and treatments, 12-well plates containing different MS concentrations (1x, 0.5x, or 0.25x) with or without salt stress (75 mM NaCl) were used in a different experiment. The consecutive steps were the same than previously described.

To analyze the effect of biostimulants, a preliminary experiment using *Arabidopsis* seedlings supplemented with different concentrations of GABA (Sigma-Aldrich, Inc.) was performed using the same sowing conditions. Three-days old *Arabidopsis* seedling were then transferred into 24 multi-well plates containing 1x MS medium with/without three concentrations (0.1, 1 or 10mM) of the compound and four different growth conditions: control and three concentrations of NaCl (50, 75 or 100 mM).

The HTS *Arabidopsis* method was improved using 48-well plates for characterizing biostimulants. Putrescine, Spermidine, Spermine and Proline (all purchased from Sigma-Aldrich, Inc.) were used for seed priming agents. Seeds were placed on square plates with MS individually supplemented with those compounds at four concentrations (0.001, 0.01, 0.1, or 1 mM). Three days old seedlings were transferred into 1x MS medium with 0, 75 or 150 mM NaCl solution.

Additionally, a HTS method based on the emergence of maize was established and used for the characterization of plant biostimulants. Seeds were imbibed with distilled water (controls) or with the three previously selected PAs in three concentrations (0.01, 0.1 or 1 mM) for 16 hours at 4ºC in the dark. Meanwhile, nursery trays TEKU JP 3050/160 T were filled with soil substrate (Substrat 2, Klassmann Deilmann, Geeste, Germany) and cut to fit into hydroponic inserts for standard PlantScreen^{TM} measuring trays (Photon Systems Instruments, Brno, Czech Republic). The cut trays had 110 cells (one cell equals to 21.5 mL). Thereafter, one seed per cell was sown 1 cm deep into the substrate. Each tray was watered to its full capacity with tap water or with a solution of NaCl at two concentrations: 75 mM NaCl or 150 mM NaCl. Afterwards, all trays were watered using 0.5 L of tap water every third day until the end of the experiment. The trays were assigned to the control, moderate salt stress, and severe salt stress groups randomly at the beginning of the experiment and the experiment was repeated twice over different days to evaluate the reproducibility of the bioassay.

- **Phenotyping platform, experimental setup and assay conditions.**

The two developed HTS methods were performed onto the OloPhen platform that uses the PlantScreen^{TM} XYZ system installed in a growth chamber with a controlled environment and cool-white LED and far-red LED lighting (Photon Systems Instruments, Brno, Czech Republic). The conditions were set to simulate a long day with a regime of at 22ºC/20ºC in a 16/8 h light/dark cycle, an irradiance of 120 µmol photons of PAR m^{-2} s^{-1} and a relative humidity of 60%. The PlantScreen^{TM} XYZ system consists of a robotically driven arm holding an RGB camera which is automatically moved above the plates to take RGB images (resolution 2500 x 2000 pixels) of single plates (for the well-plates) or a tray (for maize emergence test) from the top view. The imaging of each 12 and 24 multi-well plate was performed once per day and each 48 well plate was performed twice per day (at

10 a.m. and 4 p.m.) for 7 days. For recording maize emergence, RGB images were taken once every two hours over 5 days and the time of emergence was set as the first imaging time when the seedling was already visible. Some of the seedlings may not have emerged at all until the end of the experiment. For these, the total duration of the experiment was recorded and they were denoted as "censored".

The data of all experiments were automatically stored in PlantScreen XYZ database, exported by PlantScreen Data Analyzer software and analyzed using an in-house software routine implemented in MatLab R2015.

- **Biometric parameters.**

The changes in green area (pixels) were measured twice per day in each *Arabidopsis* seedling using the aforementioned automatic system. The relative growth rate (RGR) per hour or day was estimated for each replicate and variant.

- **Determination of leaf color in *Arabidopsis* rosette under control and salt stress conditions.**

For non-invasive estimation of the changes in leaf color, three vegetative indices (NGRDI, GLI, and VARI) were calculated which correlate with the plant biomass, nutrient status and tolerance to abiotic stress [24,25]. The values corresponding to particular color channels (red = R, green = G, and blue = B) were extracted for each pixel within the plant mask, and the vegetative indices were calculated.

Subsequently, indices representing particular seedlings were determined by calculating the mean values for each plant mask. The mean value for each 48-well plate was then calculated.

- **Statistical analysis and data presentation.**

To assess the differences between the projected areas (pixels) of two or more groups of plants at a particular time-point, the non-parametric Kruskal-Wallis one-way analysis of variance by ranks and the parametric one-way analysis of variance (ANOVA) were used. When ANOVA was significant, the differences were determined using the Dunn-Sidák's

correction. For analysis of multidimensional data, visual representations created using the MatLab R2015 software, were used. The relationship among traits was analyzed via Pearson's correlation and the significance of the regression was determined by applying a Student's t-test to the linear curves and after linearization of non-linear curves.

For seedling emergence bioassay, the nonparametric log-rank test was used to test the difference in seedling emergence among various subgroups. However, this test it is not suitable for capturing differences in various aspects of the emergence process. The emergence of maize seedlings was then analyzed by fitting the Gompertz curve to the empirical cumulative distribution function.

IV. Results

Interactions involved in plant responses to stress conditions.

In depth study was carried out on up to date information on the GABA pathway, synthesis and catabolism, and further analyses of the interactions involved in plant responses to stress conditions, suggesting highly conserved pathways connecting primary and secondary metabolism, with an overlap of regulatory functions related to stress responses and tolerance among phytohormones, AAs and PAs.

Standardization of the bioassay for HTS of *Arabidopsis* rosette growth in normal and stressed conditions.

❖ Bioassay optimization and validation.

To define the most suitable screening conditions, 12- and 24-well plates were prepared following the experimental protocol with nine and six replicates per variant and analyzed for 9 days. In both cases there is a similar profile, showing highly significant exponential growth. Further, the relative growth rate shows the same tendency but with higher values for those grown in 12-well plates. The Kruskal–Wallis test was used to evaluate statistically significant differences in rosette area. Finally, to test the reliability of the method, we compared the green area estimated by automated RGB imaging with the weight of the rosettes determined manually, obtaining a highly significant correlation among the two parameters (R= 0.94 and 0.85).

❖ Standardization of control conditions for the bioassay.

To select our standardized normal conditions, we tested whether MS concentration and the addition or not with sucrose influenced *Arabidopsis* rosette growth. A clear concentration-dependent increase in rosette area was found, indicating that 1xMS is the best growing medium for *Arabidopsis* seedlings *in vitro*. The presence of sucrose did not induce significant differences in the growth. Therefore, the use of 1xMS without sucrose was determined as the standard growing medium for our assay.

❖ Use of the bioassay in the salt-stress studies.

An experiment to test the effect of salt stress on *Arabidopsis* rosette growth was performed using 1x MS medium supplemented with 50, 75, 100, or 150 mM NaCl. Three replicates of 24-well plate were used for each tested variant, with no significant differences among them. Both time-dependent increase in shoot area and RGR were negatively affected by NaCl treatment in a dose-dependent manner. 100 mM or 150 mM NaCl showed a very dramatic growth inhibition and fast senescence. Overall, these results proved the potential of the assay to be used as a tool for salt-stress studies.

Finally, to evaluate the use of our HTS method for testing biostimulants, *Arabidopsis* plants were treated with GABA with three different concentrations (0.1, 1 or 10mM) under control and salt stress conditions (50, 75 or 100 mM NaCl). Seedlings grown on media supplemented with GABA does not induced significant differences in *Arabidopsis* rosette growth. Consequently, GABA was discarded as a potential compound to be use as plant stress mitigator.

- **HTS of *Arabidopsis* rosette growth as a suitable assay for the characterization of biostimulants under control and salt stress conditions.**

Previous HTS protocol was optimized using 48 well plates with four biological replicates randomly distributed in the platform. The seedlings were measured twice per day (at 10:00 h and at 16:00 h) for 7 days, observing as a negligibly difference in the green area among replicates and a similar RGR. The seed batch was separated into three different size categories: 250–280, 280–300, and >300 µm, and given their abundance and good growth performance, 280–300 µm seeds were selected as the standard for subsequent experiments.

To test our HTS method for characterizing biostimulants, *Arabidopsis* seeds were primed with three PAs (Put, Spd, Spm) and the AA Pro over the concentration range (0.001, 0.01, 0.1, or 1 mM). The results differences in the mode of action for the four compounds applied to *Arabidopsis* rosette growth. Put and Spd were identified as plant growth promotors and stress alleviators, whereas Spm was less efficient and, can therefore be classified as a plant growth promotor rather than a stress alleviator. Seed priming using Pro was less effective than with PAs, and the most positive effect was under a severe salt stress.

❖ Effect of biostimulants on *Arabidopsis* seedling establishment.

The green area of the *Arabidopsis* seedlings immediately after the transfer into 48 well plates was analyzed under control conditions. The seed priming significantly affected the *Arabidopsis* rosette size, pointing at our method can record traits in a complex manner that describes the effect of priming on all important stages of early development.

❖ Effect of biostimulants on leaf color of *Arabidopsis* rosettes under control and salt stress conditions.

Three leaf color index of *Arabidopsis* rosettes were also calculated using the RGB data. Among them, GLI exhibited the highest sensitivity to salt stress. The seed priming with Put and Spd generated the highest greenness under control and salt stress conditions.

❖ PBC Index for estimating the biostimulant mode of action.

Plant Biostimulant Characterization (PBC) index was developed and represented up to four traits: seed germination rate (%), seedling establishment (green pixels after transfer into 48 well plates), growth capacity (pixels) and the leaf color index (GLI) for the primed and non-primed seeds. Put was the most efficient seed priming agent working as plant growth promotor and stress alleviator. The remaining compounds exhibited a concentration and growth-condition-dependent response. The presented MTHTS approach is an adequate tool for fast and simultaneous analysis of various concentrations and growth conditions for identification and, especially, characterization of the mode of action associated with new biostimulants.

- **CroSeEm as HTS of maize emergence for characterizing priming agents in control and salt stress conditions.**

Crop Seedling Emergence (CroSeEm) is a HTS bioassays that monitors automatically the first appearance of the coleoptile in maize under control and saline conditions.

❖ Setup for CroSeEm analysis.

The emergence of maize seedlings was analyzed by the parametric method Gompertz curve. From the curve, three traits (final germination rate, time lag and emergence synchronicity) were extracted. We observed a delay in the speed of maize seedling emergence compared to the control in two independent experiments with 75 mM NaCl. This trait, together with the time lag, was particularly affected when 150 mM NaCl was used. However, the final emergence rate was almost unchanged, suggesting that this trait was less of a stress indicator. The three extracted traits should be independently analyzed. Thus, analyzing them separately is a more sensitive and reproducible approach for the characterization of maize emergence under salinity.

❖ Characterization of priming agents.

Maize seeds primed with PAs did not affect the final emergence rate under any growth conditions. However, under salt stress the effect of the PAs becomes visible, with Put as the most efficient treatment.

V. Conclusions

The present thesis address the development of reproducible HTS bioassays to be used for selecting and characterizing biostimulants and their modes of action under different salt stress concentrations.

- For this purpose, in depth review about the plant response to stress was performed. It pointed to the existence of a highly conserved pathway expressed in plants under stress, in which the crosstalk of phytohormones, PAs and/or GABA define plant stress tolerance. The mode of action of these compounds strongly suggest them as potential candidates to mitigate the adverse effects of multiple stresses.
- The development and optimization of HTS method based on *Arabidopsis* rosette growth in multi-well plates for the characterization of biostimulants mode of action was achieved. Based on their contribution to the plant development and stress tolerance, their mode of action could be define such as plant growth promotor/inhibitor and/or stress alleviator.
- In order to create strategies for improving crops, a HTS method of seedling emergence "CroSeEm" was developed. It is suitable for characterizing different maize lines and/or seed priming agents against salinity.
- Overall, it was demonstrated that the use of PAs as seed priming agents can be a useful biotechnological practice to improve salt stress response of plants.

In summary, we consider that the new protocols based on HTS methods could make easier and faster the identification of the mode of action for known biostimulants and help in the identification of new ones.

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VII. List of author´s papers

- Kateřina Podlešáková*, **Lydia Ugena***, Lukáš Spíchal, Karel Doležal, Nuria De Diego. "Phytohormones and polyamines regulate plant stress responses by altering GABA pathway". *New Biotechnology;* vol.48, pp.53-65, 2019.

- Nuria De Diego, Tomáš Fürst, Jan F. Humplík, **Lydia Ugena**, Kateřina Podlešáková, Lukáš Spíchal. "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*, vol.8, Art. 1702, 2017.

- **Lydia Ugena***, Adéla Hýlová*, Kateřina Podlešáková, Jan F. Humplík, Karel Doležal, Nuria De Diego and Lukáš Spíchal. "Characterization of biostimulant mode of action using novel multi-trait high-throughput screening of *Arabidopsis* germination and rosette growth". *Frontiers in Plant Science*, vol.9, Art. 1327, 2018.

- **Lydia Ugena**, Jan F. Humplík, Tomáš Fürst, Nuria De Diego, Lukáš Spíchal. "CroSeEm: a high-throughput emergence assay for screening maize seedlings under salinity" *(under revision).*

- Cintia F. Marchetti*, **Lydia Ugena***, Jan F. Humplík, *et al.* A novel image-based screening method to study water deficit response and recovery of barley populations using canopy dynamics phenotyping *Frontiers in Plant Science (under revision).*

- Jan F. Humplík, Jakub Dostál, **Lydia Ugena**, *et al.* Time-to-event data in plant biology: the case for Bayes. *Nature Plants (under revision).*

- Ameztoy, K.; Baslam, M.; Sánchez-López, Á. M. *et al.* "Plant responses to fungal volatiles involve global post-translational thiol redox proteome changes that affect photosynthesis" *Plant, Cell and Environment (under revision).*

VIII. Conference attendance.

- **Ugena L.**, Sánchez-López A.M., Tarkowská D., *et al*. "*Alternaria alternata* volatiles modify hormone profile for inducing growth and starch accumulation in *Arabidopsis"*. Novel approaches to unravel the plant-soil-microbial systems in action. Piacenza, Italy, 2016.
- **Ugena L**., Sánchez-López A.M., Bahaji A., *et al*. "VOCs emitted by diverse microorganisms modify plant development through cytokinin action". Growth Regulators on the way 2016. Mala Moravska, Czech Republic, 2016.
- Sánchez-López A. M.*, Bahaji A. *, De Diego N.*, *et al.* "*Arabidopsis* responds to volatile compounds emitted by phytopathogenic microorganisms through plastidic phosphoglucose isomerase independent mechanisms". Plant Biology Europe. EPSO/ FESPB. Prague, Czech Republic, 2016.
- De Diego N., Fürst T., Humplík J.F., **Ugena L.,** Podlešaková, Spíchal L. "High-throughput screening of *Arabidopsis* shoot growth in multi-well plates". Plant Organ Growth Symposium, Elche, Spain, 2017.
- **Ugena L.,** De Diego N., Podlešáková K., *et al*. "Identification of biostimulants under different growth conditions using high-throughput *Arabidopsis in vitro* bioassay". Growth Regulators on the Way, Kouty nad Desnou, Czech Republic, 2017.
- **Ugena L.,** De Diego N., Podlešáková K., *et al.* "High-throughput *Arabidopsis in vitro* bioassay for identification of biostimulants". Trends in Natural Product Research. Natural Products in Health, Ago-food and Cosmetics. Lille, France, 2017.
- **Ugena L.,** De Diego N., Podlešáková K., *et al*. "Screening for identification of biostimulants using high-throughput *Arabidopsis in vitro* bioassay". Plant Biotechnology Meeting: Green for Good IV. Olomouc, Czech Republic, 2017.
- Spíchal L., De Diego N., Humplík J. F., Füsrt T., **Ugena L.,** Podlešáková K. "Identification of biostimulants and their mode of action using automated high-throughput bioassaying and phenotyping". The 3rd World Congress on the use of Biostimulants in Agriculture. Miami, United States 2017.
- De Diego N., Novák O., **Ugena L**., Doležal K. "Metabolite determination and quantification in different biostimulants using UHPLC-MS/MS". The 3rd World Congress on the use of Biostimulants in Agriculture. Miami, United States, 2017.

- **Ugena L.**, Hýlová A., Podlešáková K., *et al.* "Characterization of biostimulants using high-throughput screening in different crops under abiotic stress". Chemistry and Biology of Phytohormones and Related Substances. Luhačovice, Czech Republic, 2018.
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- **Ugena L**., Podlešaková K., Bryksová M., *et al*. "The crosstalk between phytohormones and polyamines regulate plant stress tolerance". Auxins and Cytokinins in Plant Development and interaction with other phytohormones. Prague, Czech Republic, 2018.
- **Ugena L.**, Hýlová A., Podlešáková K., *et al* "Characterization of biostimulants using multi-trait high-throughput screening in *Arabidopsis* under abiotic stress". XXXII Reunión Argentina de Fisiología Vegetal (RAFV) y XV Congreso Latinoamericano de Fisiología Vegetal. Córdoba, Argentina, 2018.
- **Ugena L.** Almagro G., Sánchez-López A.M., Pozueta- Romero J., De Diego N., Gruz J. "Identification of biologically active substances from different types of biostimulants in the interaction with the model plant *Arabidopsis thaliana".* Chemistry and Biology of Phytohormones and Related Substances. Luhačovice, Czech Republic, 2019.
- **Ugena L.**, Humplík J. F., Fürst T., et al. "High-throughput emergence assay for screening of potential biostimulants mode of action that improve crops tolerance to salinity". Plant Biotechnology Meeting: Green for Good V. Olomouc, Czech Republic, 2019.
- **Ugena L.**, Hýlová A., Podlešáková K., *et al.* "Characterization of biostimulants using novel high-throughput screening approaches in plants under different stress conditions". XIII Meeting of the Spanish Society of Plant Physiology and the XVI Hispano-Portuguese Congress of Plant Physiology. Pamplona, 2019.