High-throughput automated phenotyping as a shortcut to more effective biostimulants: from seeds to crops

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Background

Development of highly effective biostimulants requires an accurate evaluation of the effects of candidate products on morpho-physiological traits of selected crops during different developmental stages and environmental conditions. As conventional screening methods are time consuming, destructive, labour intensive and expensive, high-throughput plant phenotyping procedures were recently proposed as effective and high-precision tools for product screening. In this work, we evaluate the effects of both newly-formulated and commercial biostimulants as growth promotors and/or stress alleviators on plants subjected to abiotic stresses (drought and salinity) at different phenological stages. Using three different PlantScreen™ systems developed by PSI (Photon Systems Instruments, Drasov, Czechia), we were able to monitor the properties of the selected substances, given either as seeds coating, foliar spray or soil drenching, on germination of wheat seeds and growth and photosynthetic performance of in-vitro Arabidopsis seedlings (Robotic XYZ System), lettuce (Compact System) and tomato (Modular System).

From seeds...

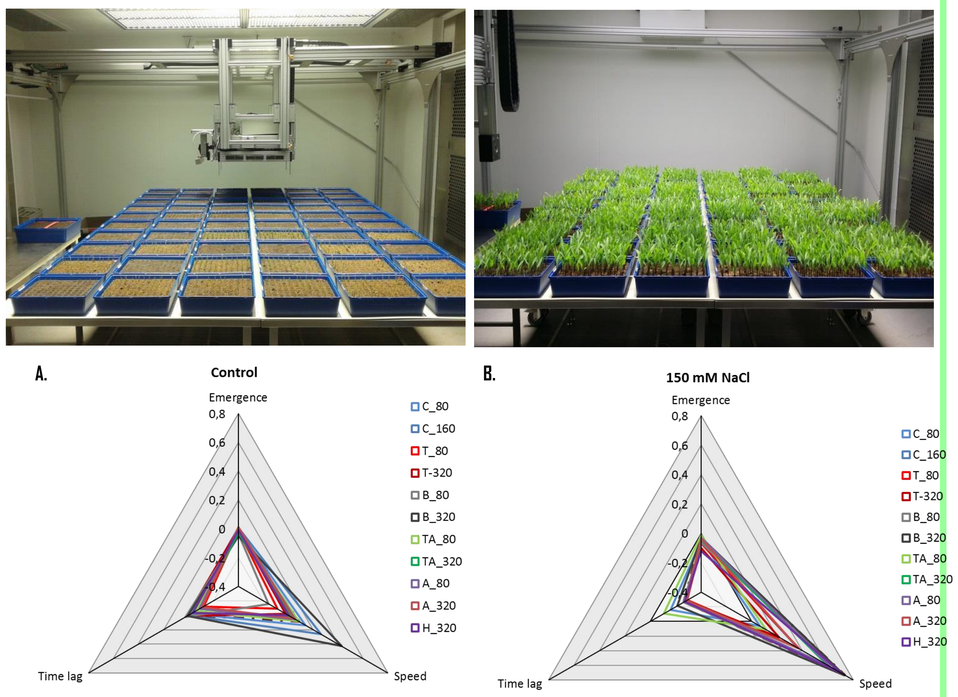
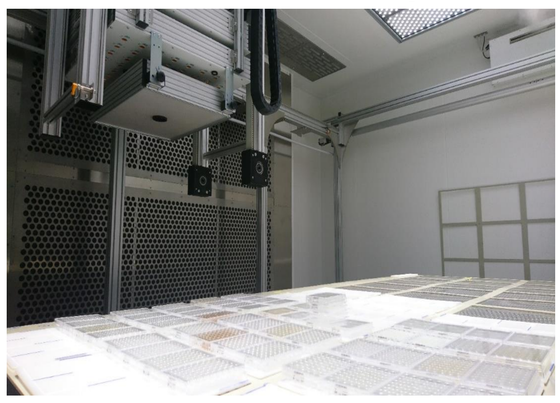


Figure 1| Experimental setup at the beginning (left) and at the end of the experiment (right).
Figure 2 (A-B)| Radar graphs picturing the Plant Biostimulant Characterization (PBC) index (ratio between coated seeds and control ones) for the **final emergence rate**, the **time lag** (the amount of time occurring between the germination of 5% control and coated seeds) and the **speed of germination**, both in control (A) and salt-stress conditions (B). For the index calculation, the differences between the controls of the different growth conditions and variants (compound and concentration) under the same conditions were calculated as the log2 of the ratio.

Summary

The seeds coated with the protein hydrolysate "B" in the highest concentration has proven to improve the overall germination performance (+49 % if compared to control) of the seeds both in control and salt-stressed conditions, especially for the speed parameter. On the opposite, the protein hydrolysate "T" has reduced the values of all the three parameters, if compared to control.

PlantScreen™ XYZ System

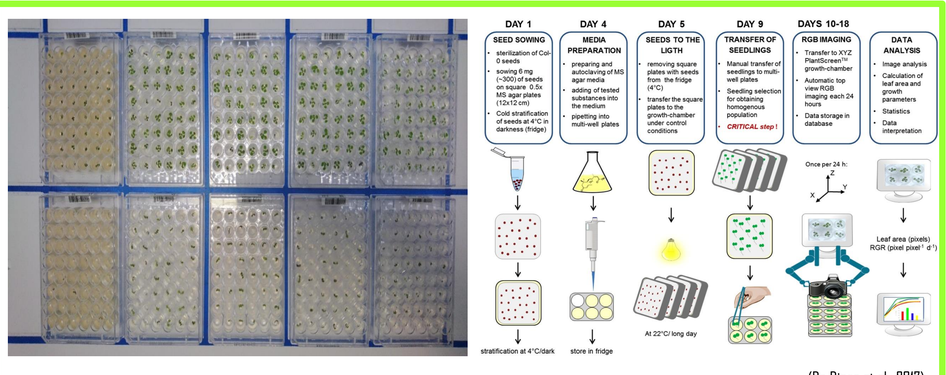


Material and methods

Triticum aestivum seeds, coated with 6 biostimulants at different concentrations, were sown into soil fully soaked either with tap water or 150 mM NaCl solution. The environmental conditions were 22◦C/20◦C in a 16/8 h light/dark cycle, an irradiance of 120 µE of PAR and a relative humidity of 60%. Starting from the first measure, RGB pictures were taken every second hour over the twelve days of the experiment. For each tray, **final emergence rate** in the last day of measurement, **time lag** and **speed or synchronicity of emergence**.

The plates were filled in with normal MS or with MS enriched with 150 mM NaCl solution; 5 different biostimulants at growing concentration (from 0,001 to 1 µl/ml) were then added to each plate. An Arabidopsis seedling were transplanted in each well. The environmental conditions were 22°C/20°C 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%. After the first RGB imaging, two images per day were taken for a period of 8 days. For each plate, the **growth rate** and the **percentage of survival** was monitored.

...to seedlings...



(De Diego et al., 2017)

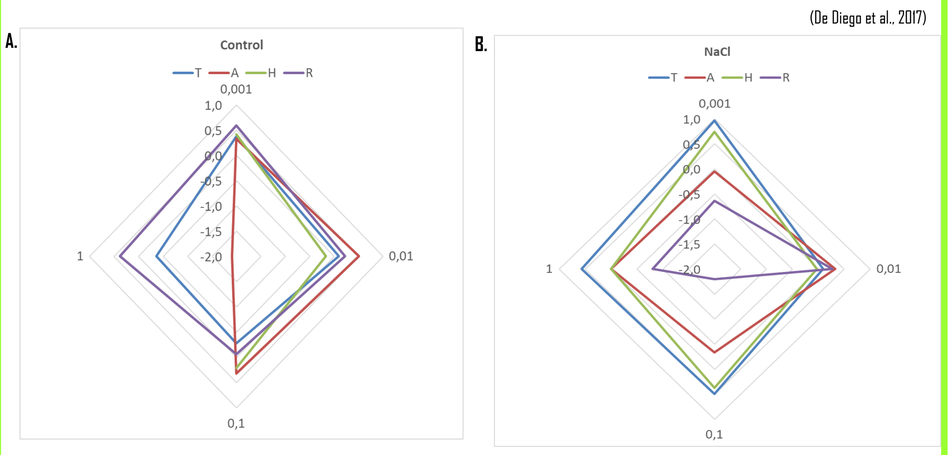


Figure 3. | Experimental setup (left) and scheme (right) of the trial.
Figure 4 (A-B). | Radar charts of the Plant Biostimulant Characterization (PBC) index of plants in control (A) and salt enriched media (B), calculated by summing the relative changes (log2) obtained for the growing parameters for each compound (T, A, H or R) and concentration (0.001, 0.01, 0.1 and 1 mM).

Summary

The PBC index shows that the microbial metabolites solution "R" in the lowest concentration is improving plantlets growth in control conditions, whereas in salt stress condition the most performing one was the plant hydrolysate "T", once again in the lowest concentration.

...to leafy crops...

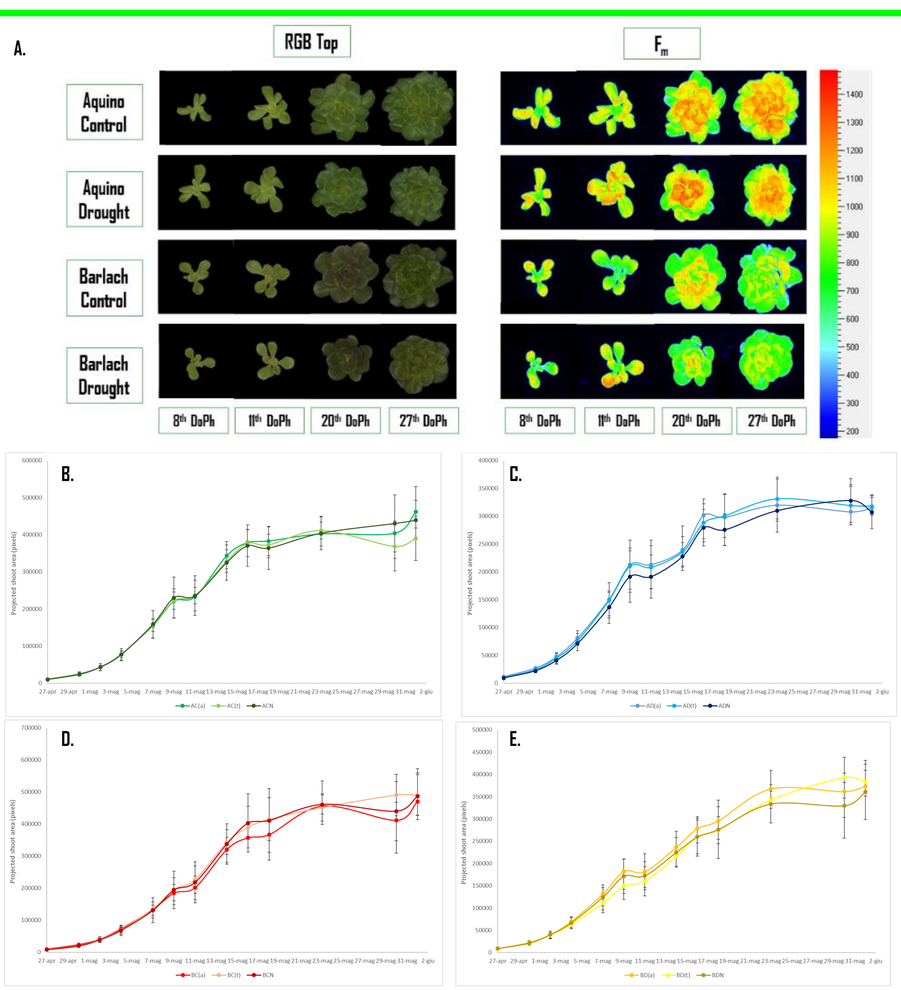
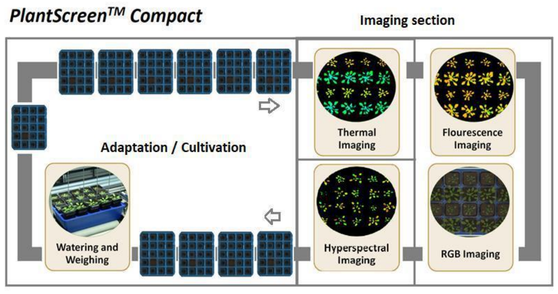


Figure 5(A-E)| RGB top images and ChlFI images of F_m (maximum fluorescence) in false colors for control and drought stressed plants (A) (DoPh = Day of Phenotyping). Area increase for Aquino plants in control (B) and stressed conditions (C) and for Barlach plants in control (D) and stressed conditions (E).

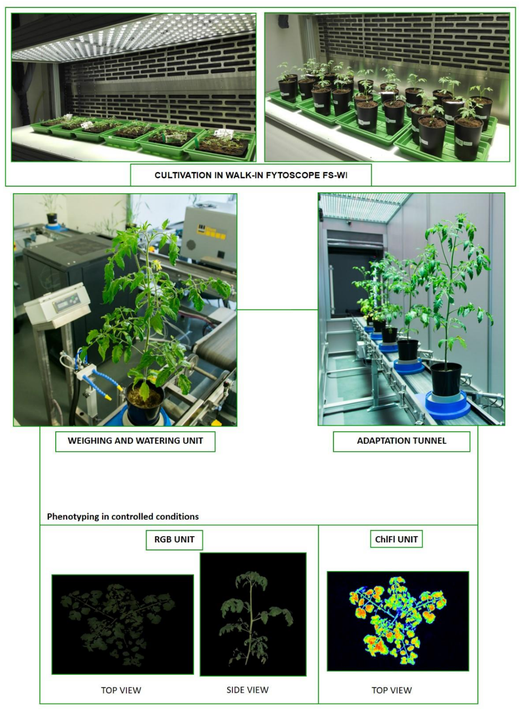
Summary

A reduction in projected top area measured in number of green pixels is present in both cultivars as consequence of drought stress; however, the biostimulants applied did not have any relevant effect, neither in control or stress conditions.

PlantScreen™ Compact System



PlantScreen™ Modular System



...to vegetable crops.

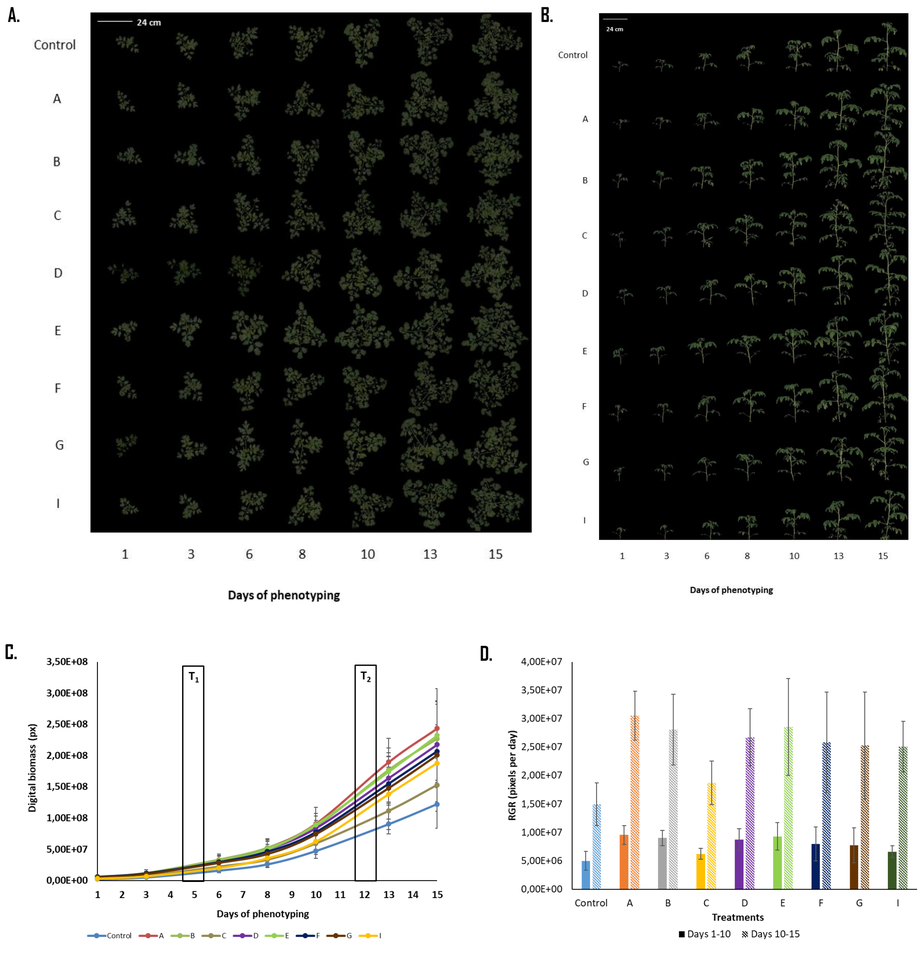


Figure 6(A-D)| Top (A) and side view (120◦) (B) RGB image of the tomato plants over the time of phenotyping period. Digital biomass quantified over time of phenotyping period (C) and Relative Growth Rate (D). Values represent the average of six biological replicates per treatment. Error bars represent standard deviation. T1 and T2 correspond to days of protein hydrolysate application.

Summary

Biostimulant application always produced an increase in biomass acciumulation, with the «A» biostimulant proving to be the most effective.

Material and methods

Seeds of two cultivars of *Salanova®* ettuce (Aquino and Barlach) were grown into a Growth Chamber (**Walk-in FytoScope FS-WI**), at 23°C day and 19°C night, 60% relative humidity and 12h light-12 h dark regime (250 µE white light, 5.5 µE far-red light). They were equally divided into two groups: **control** (watered up to 70% of Soil Water Content) and stressed plants (40% of Soil Water Content). Two different biostimulants of vegetal origin have been applied once a week via foliar spray for 5 times to half of the plants. For 5 weeks, the biomass accumulation and the photosynthetic efficiency of the plants has been monitored using the **PlantScreen™ Compact System.**

Material and methods

Plants of *Solanum lycopersicum* L. Hybrid F1 CHICCO ROSSO were transplanted into 3L pots filled in with a mixture of Klasmann 2 substrate and river sand in 3:1 ratio and grown into a Growth Chamber (**Walk-in FytoScope FS-WI**) at 22°C, 60% Rh and 250 µE White Light with 16h/8h light/dark regime. Plants were distributed into 14 groups with 6 replicates per group; 8 biostimulants were given twice as foliar spray. Throughout the experiment, growth photosynthetic efficiency and temperature were measured on plants using **PlantScreen™ Modular System.**

Acknowledgements

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Conclusions

Precise and accurate assessment of phenotypic variables is critical for unravelling and quantifying the biostimulant activity of various products. High-throughput phenotyping technologies proved to be effective for product screening and development as efficient means to automated, non-destructive monitoring of multiple morpho-physiological plant traits. The time-series measurements allow to follow the progression of growth, plant performance, and stress responses of individual plants at high-resolution, providing information about the varying effects of the substances on plants, depending on growth conditions (with some biostimulants acting as growth improvers, others as stress alleviators), application method, concentration of the substance (that could become phytotoxic) and developmental stage.
High-throughput phenotyping could contribute not only to accelerate the selection of substances to be used as biostimulants, but also to provide information on the most effective method and timing for the application.

References

De Diego N, Fürst T, Humplík JF, Ugena L, Podlešáková K and Spíchal L (2017). An Automated Method for High-Throughput Screening of Arabidopsis Rosette Growth in Multi-Well Plates and Its Validation in Stress Conditions. Front. Plant Sci. 8:1702.
Paul K, Sorrentino M, Lucini L, Rouphael Y, Cardarelli M, Bonini P, Reynaud H, Canaguier R, Trtílek M, Panzarová K and Colla G (2019) Understanding the Biostimulant Action of Vegetal-Derived Protein Hydrolysates by High-Throughput Plant Phenotyping and Metabolomics: A Case Study on Tomato. Front. Plant Sci. 10:47.
Paul K, Sorrentino M, Lucini L, Rouphael Y, Cardarelli M, Bonini P, Miras Moreno MB, Reynaud H, Canaguier R, Trtílek M, Panzarová K and Colla G (2019) A Combined Phenotypic and Metabolomic Approach for Elucidating the Biostimulant Action of a Plant-Derived Protein Hydrolysate on Tomato Grown Under Limited Water Availability. Front. Plant Sci. 10:493
Rouphael Y, Spíchal L, Panzarová K, Casa R and Colla G (2018) High-Throughput Plant Phenotyping for Developing Novel Biostimulants: From Lab to Field or From Field to Lab? Front. Plant Sci. 9:1197. doi: 10.3389/fpls.2018.01197
Ugena L, Hýlová A, Podlešáková K, Humplík JF, Doležal K, De Diego N and Spíchal L. (2018) Characterization of Biostimulant Mode of Action Using Novel Multi-Trait High-Throughput Screening of Arabidopsis Germination and Rosette Growth. Front. Plant Sci. 9:1327.