USING AUTOMATED HIGH-THROUGHPUT PHENOTYPING TO QUANTIFY AND VISUALIZE EARLY STRESS RESPONSES IN PLANTS

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Introduction

The major challenge the global agriculture and plant biology field is nowadays facing in order to sustain the global food security is the identification of new high-yielding genotypes of agricultural crops that are adapted to our future climate. The use of automated high-throughput phenotyping approaches offers the suitable tool for accelerating crop breeding progress by linking the selected gene function with phenotypic traits in relevant environment. Photon Systems Instruments (PSI), Ltd developed a range of PlantScreen™ plant phenotyping platforms for the greenhouse, growth room and field for high-resolution and high-throughput quantification and visualisation of phenotypical signatures that might be informative of plant development, physiological status, and performance in given conditions. These incorporate imaging technologies for RGB and morphometric analysis, thermal analysis, hyperspectral analysis and, critically, in-depth analysis of chlorophyll fluorescence kinetics. The latter technique, as a rapid tool for monitoring photosynthetic processes, is key to the identification of early onset of stress, and recovery from stress after acceleration.

Here we used the PlantScreen™ Conveyer System platform to visualize and quantify early plant response to stress by analysing plant structure, growth, and physiological status such as photosynthetic performance after application of glyphosate, nonselective, broad-spectrum, post-emergence herbicide.

Methods

For quantitative kinetic investigation of glyphosate mode of action in Arabidopsis thaliana PlantScreen™ Conveyer high-throughput phenotyping platform was used for parallel time-course hyperspectral image analysis, morphometric analysis and in-depth analysis of chlorophyll fluorescence kinetics. Plants were grown in 10h-12h light conditions under cool-white LED illumination of 600 µmol m⁻² s⁻¹ in Walk-in Phytoclimatic Dumbell (PSI). 72 days old plant were sprayed with 0.5% seaweed (control), 0.5% and 2 % glyphosate (v/v) (Rendama, Monsanto). IF replicates were used for each variant. Immediately after the spraying the phenotyping analysis in high-throughput PlantScreen™ Conveyer System was initiated. RGB structural imaging, chlorophyll fluorescence and VNIR hyperspectral imaging was acquired each 15 min for next 72 hours. For automated image processing, data analysis and visualisation PlantScreen™ Software tool package was used.

Results

Fig. 1A) Visible top view RGB images demonstrate that around 25 hours from glyphosate application (0.5%, 0.2% R) yellowing of leaves occurred. Lower effect on growth retardation is visible in the acquired images. B) Quantitative morphometric data clearly demonstrate that growth dynamics of the plants is impaired around 25 hours after glyphosate application.

Fig. 2A) Screening induction experiment measured in PAM mode was used to determine photosynthetic performance of the plants following the glyphosate treatment. A) Decline in maximum PSII quantum yield in dark-adapted state (Fv/Fm) in glyphosate treated plants was observed around 5-6 h after spraying. B) Fluorescence parameters as Fv/Fm (fluorescence decline ratio used to assess plant vitality) and FνR (non-photochemical quenching as process used by plants to protect themselves from high-light) were affected in glyphosate-treated plants already 3 hours after application.

Conclusions

Rapid characterization of plant structural and functional dynamics by using high-throughput non-invasive phenotyping approaches has the power to significantly speed up the process of identification of desired photosynthetic traits and accelerate crop breeding progress by linking the selected gene function with phenotypic traits in relevant environment.

We have used PlantScreen™ Conveyer high-throughput phenotyping platform to characterise early mode of action of herbicide glyphosate. To identify early stress response of Arabidopsis thaliana to glyphosate application parallel time-course hyperspectral image analysis, morphometric analysis and in-depth analysis of chlorophyll fluorescence kinetics was performed. Our data clearly demonstrate the power of chlorophyll fluorescence kinetic analysis as it allows to differentiate effect of glyphosate on photosynthetic performance of plants already 3 hours after herbicide application. By using VNIR hyperspectral imaging we show that 12 hours after decline of Rfd parameter, chlorophyll content in herbicide treated plants decreased (NOVI). Results of morphometric analysis show that growth dynamics of stressed plants in impaired 23 hours after the treatment.

Acknowledgements: This work was carried out at Photon Systems Instruments (Czech Republic) with partial financial support through HAVROST FFT Marie Curie Initial Training Network.


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