High-throughput screening tools for identification of traits contributing to salinity tolerance in *Arabidopsis thaliana*

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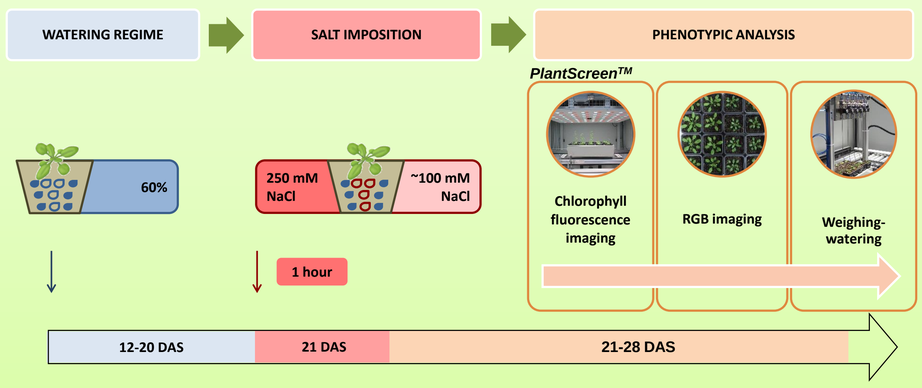
Introduction

Recently developped approaches in the field of high-throughput image-based phenotyping approved the importance of automated non-invasice phenotyping tools for unravelling the complex questions of plant structural and functional phenotypes in controlled or dynamically changing environment. Soil salinity is one of the main stress factors that are severely affecting the agriculture land in global scale and results in significant reduction of plant growth and yield. It was shown that plants suffer a rapid growth reduction upon the first exposure of their roots to salt stress, which is occurring prior to the accumulation of ions to toxic concentrations in the shoots. To enhance our understanding of the early responses to salinity, we designed an experimental protocol based on using high-throughput and non-invasive imaging technologies developed at Photon Systems Instruments (PSI, Czech Republic). The methodology presented is based on automated integrative high-throughput analysis of photosynthetic performance, growth analysis and color index analysis at the onset and early phase of salinity stress response in *Arabidopsis thaliana* ecotypes grown in soil. Here we show that the developped experimental procedure allows to analyse dynamically structural and physiological phenotypes early upon stress imposition by using two Arabidopsis accessions Col-0 and C24, where C24 was previsouly shown to be more resistant to salt stress. Salinity significantly and rapidly affected photosynthetic performance of the plants and impacted growth dynamics of Arabidopsis plants at different stages of stress response.

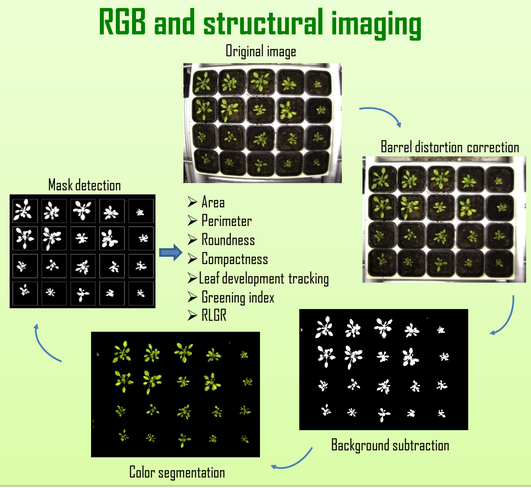
Materials and Methods

Seeds of *Arabidopsis thaliana* Col-0 and C24 accessions were germinated in 12h-12h light conditions under cool-white LED illumination of 150 µmol/m^2/s in Walk-In Phytoscope Chamber (PSI). At 7 days after stratification (DAS), seedlings were transferred to the pots with 60g of sieved soil watered to full saturation. Plants were further cultivated in the growth chamber until the 10-leaf stage was reached and salt stress was applied (21 DAS). Weight of the individual pots was automatically measured in PlantScreen™ Phenotyping System (PSI, Czech Republic) to adjust soil moisture to 60% of soil water capacity. When the 10-leaf stage was reached pots with plants were placed in 0 or 250 mM NaCl solution for one hour, ensuring saturation of the soil with the solution. The effective NaCl concentration in the soil after salt imposition corresponded to 100 mM NaCl. The plant salt stress responses were monitored for 7 days in PlantScreen™ Conveyer high-throughput phenotyping platform (PSI, Czech Republic) (phenotyping protocol) by parallel time-course image-based morphometric analysis and in-depth analysis of chlorophyll fluorescence kinetics. Measurements of different pixel properties including pixel count, color and intensity were obtained from RGB and fluorescence cameras. For automated image processing, data analysis and visualisation PlantScreen™ Software tool package was used.

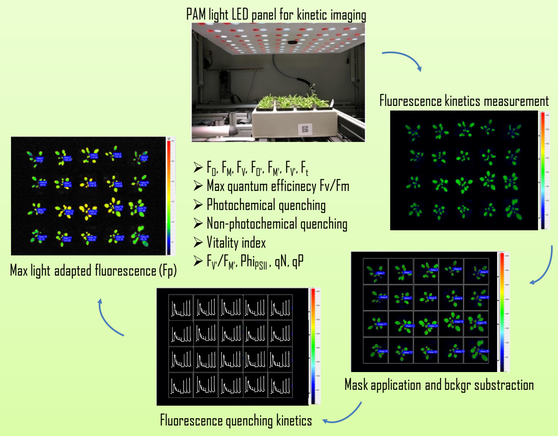
Phenotyping Protocol



RGB and structural imaging



Chlorophyll fluorescence kinetic imaging



Results and Discussion

Salinity-induced growth related responses in Col-0 and C24 Arabidopsis accessions

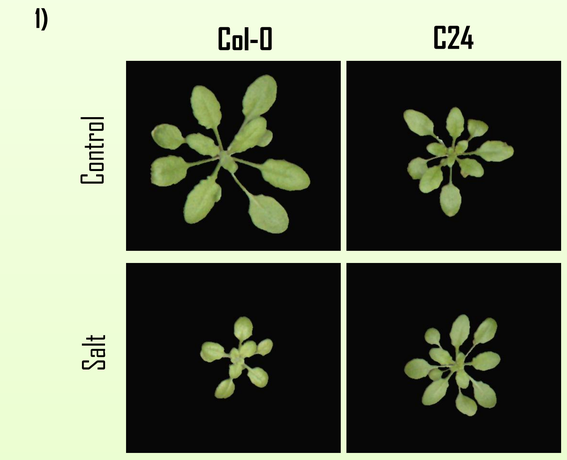


Fig.1 Representative RGB images of Col-0 and C24 accessions for control and salt stress treated plants taken at the last day of measurement (28 DAS, Day 7 after NaCl treatment). RGB/visible imaging was used quantify growth, color index and other morphological parametres in non-destructive manner dynamically during the onset of salinity to adress shoot-ion independent phase of salinity stress (Rajedran et al., 2009).

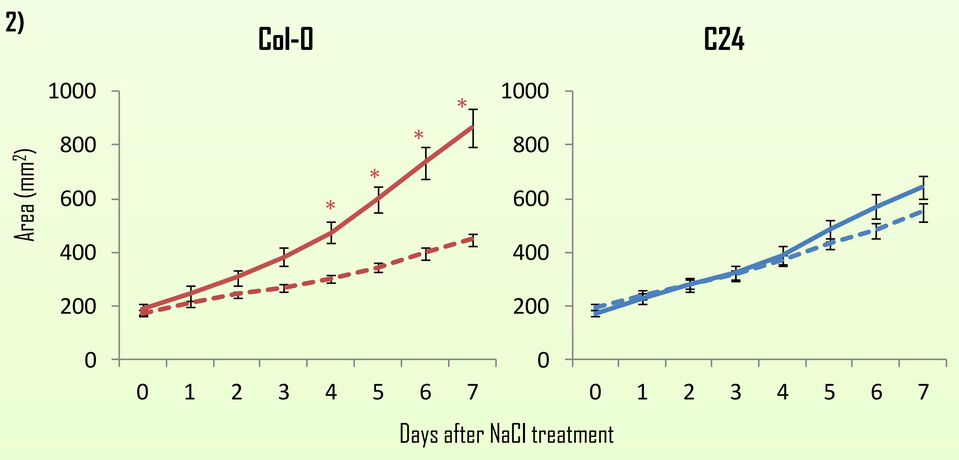


Fig.2 Growth rate in salt stress treated plants is rapidly reduced upos stress imposition. Projected rosette area in control (solid lines) and salt stress treated (dashed lines) in Col-0 (red) and C24 (blue) plants. C24 accession showed significantly lower growth reduction in response to salinity conpared to Col-0 plants, which corresponds to previously reported increased salt tolerance of C24 (Jha D. et al. 2010). The significant differences between control and salt stress treatment per accession is indicated with * for the p-value below 0.05 as calculated with one-way ANOVA. (Average ± SE, n=8 per genotype per condition).

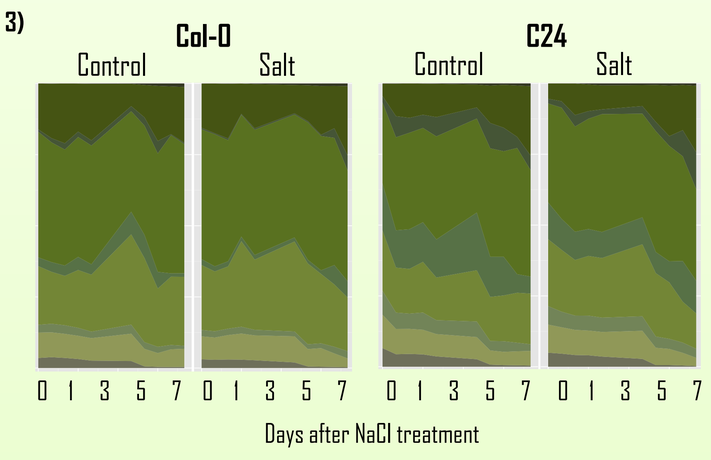


Fig.3 Relative changes in rosette color are affected by salt stress treatment. RGB images were segmented based on a calibration into nine clusters of representative green hues. The dynamic changes of the nine hues in response to the effect of salt stress. 100% stacked charts of 9 RGB color-coded greenness hues are presented as changes in percentage area over time. The greenness hues (right panel) summarize the [red:green:blue] channel values that correspond to the green hues identified through the color-segmentation process using the RGB images.

Photosynthetic performance is rapidly reduced in salt treated plants

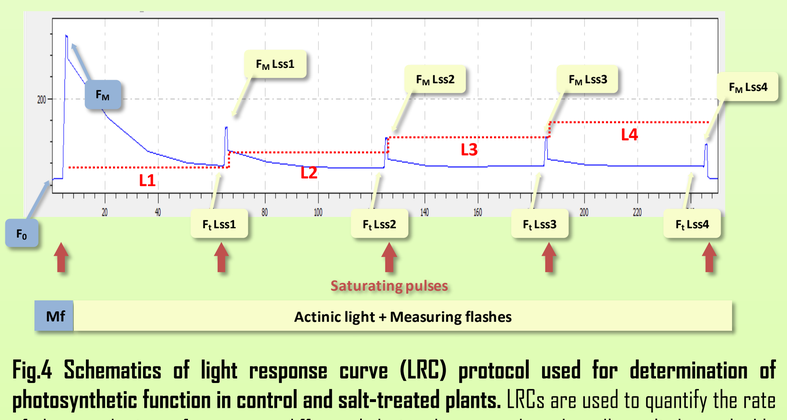


Fig.4 Schematics of light response curve (LRC) protocol used for determination of photosynthetic function in control and salt-treated plants. LRCs are used to quantify the rate of photosynthetic performance at different light irradiances and are broadly applied as valuable tool to estimate the photosynthetic light-use efficiency in response to different stresses. LRC was designed to measure quenching analysis in light adapted state at 4 light irradiances LI-L4 (from 100 to 400 µmol/m^2/s; red dotted line). Range of fluorescence parametres were calculated for different light intensities that describe photochemical and non-photochemical efficiency of photosystem II (PSII).

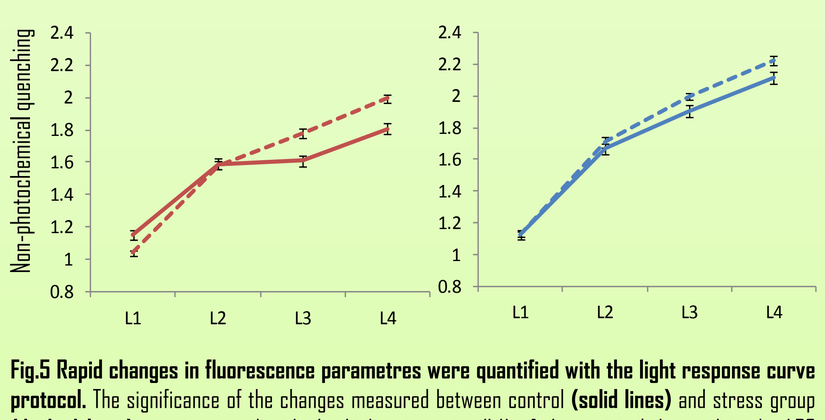


Fig.5 Rapid changes in fluorescence parametres were quantified with the light response curve protocol. The significance of the changes measured between control **(solid lines)** and stress group **(dashed lines)** was increased with the higher intensity (L4) of the actinic light used in the LRC protocol. Chlorophyll fluorescence parametres were measured for 7 days following the NaCl treatment. Changes in non-photochemical quenching (NPQ) two days after salt stress treatment are shown. NPQ refers to amount of light energy dissipated from PSII as heat. In salt treated plants the level of light induced heat dissipation increased, which correlated with decrease in photochemical quenching (data not shown), referring to fraction of open reaction centers, and with decrease of actual quantum yield of PSII photochemistry. (Average ± SE, n=8 per genotype per condition).

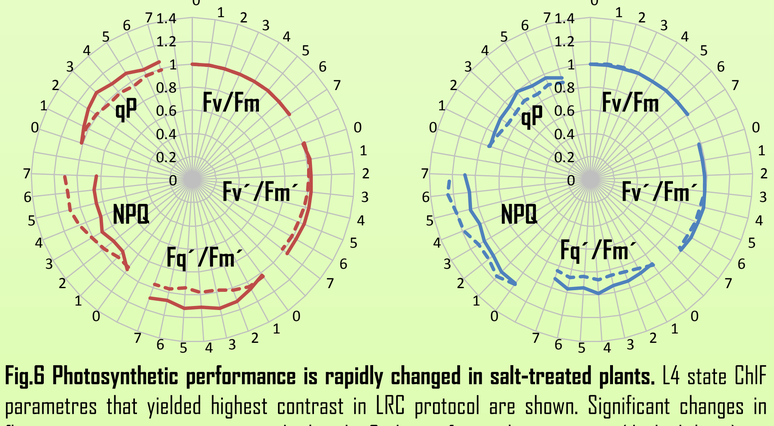


Fig.6 Photosynthetic performance is rapidly changed in salt-treated plants. L4 state ChlF parametres that yielded highest contrast in LRC protocol are shown. Significant changes in fluorescence parametres occured already 2 days after salt treatment (dashed lines) as compared to control plants (solid lines). Salinity induced rapid decrease in PSII operating efficiency (Fq´/Fm´), photochemical quenching (qP) and partially in maximum quantum yield in light-adapted state (Fv´/Fm´). Most significant changes were quantified for quantum yield of regulatory light-induced heat dissipation (NPQ), which increased already within first 24 hours upon salt treatment. Interestingly no changes following salt treatment occured for broadly used ChlF parametr Fv/Fm (Maximum quantum yield of PSII photochemistry).

Conclusions

Our work provides quantitative insights into early phase of salinity response and provides robust protocol for high-throughput image-based analysis of phenotypic traits associated with early phase of salinity response. We show that the integrative concept of PlantScreen™ high-throughput phenotyping platform provides a powerful tool for acquisition and selection of morphological andphysiological parameters, which can be used for identification of various components underlying early plant responses to environmental stress such as salinity. Rapidly after stress initiation photosynthetic performance of the salt-treated plants was compromised and followed by growth retardation and changes in greeness. In agreement with previously reported data C24 was more salt-tolerant than Col-0. The experimental protocol presented here provides robust experimental set-up for salinity tolerance screening in Arabidopsis and other plant species.

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References

Jha D, Shirley N, Tester M, Roy SJ (2010). Plant Cell Environ 33: 793–804.
Rajedran K., Tester M., Roy SJ (2009). Plant, Cell and Environment 32, 237–249