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

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Introduction

Recently developed approaches in the field of high-throughput image-based phenotyping appraised the importance of automated non-invasive phenotyping tools for unravelling the complex questions of plant structural and functional phenotypes in controlled or dynamically changing environment. Salt salinity is one of the main stress factors that are severely affecting the agricultural 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 integrated 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 developed 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 previously 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⁻¹ in Walk-In Phytoclimatic Chamber (PSI). By 7 days after stratification (DAT), seedlings were transferred to the pots with 1/3 strength of soil water to full saturation. Plants were further cultivated in the growth chamber until the 10-leaf stage was reached and salt stress was applied (21 DAT). 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 200 and 500 mM NaCl. The plant salt stress responses were monitored for 7 days in PlantScreen™ Conveyor 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 channel images. For automated image processing, data analysis and visualisation PlantScreen™ Software tool package was used.

Results and Discussion

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

Photosynthetic performance is rapidly reduced in salt treated plants

Conclusions

Our work provides quantitative insights into early phase of salinity response and provides robust protocol for high-throughput image-based analysis of photosynthetic traits associated with early phase of salinity response. We show that the integrative approach of PlantScreen™ high-throughput phenotyping platform provides a powerful tool for acquisition and selection of morphological and physiological 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 growth. 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


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 (21.03.2007). Day 7 after NaCl treatment. RGB/visible imaging was used quantified growth, color index and other morphological parameters in non-destructive manner dynamically during the onset of salinity to address shoot/vein independent phase of salinity stress (Brajková et al. 2009).

Fig 2 Growth rate in salt stress treated plants is rapidly reduced upon stress imposition. Projected routes area in control (solid lines) and salt stress treated (dashed lines) for Col-0 (red) and C24 (blue) plants. C24 accession showed significantly lower growth reduction in response to salinity compared to Col-0 plants, which corresponds to previously reported increased salt tolerance of C24 (Zhao et al. 2008). The significant differences between control and salt stress treatment per accession is indicated with * for the p-values below 0.05 as calculated with one-way ANOVA. (Average ± SD, 4-8 genotypes per condition).

Fig 3 Schematics of light response curve (LRC) protocol used for determination of photosynthetic function in control and salt-stressed plants. LRs are used to quantify the rate of photosynthetic performance of different light irradiances and were broadly applied as a 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 (14 LRC to 1400 µmol m⁻² s⁻¹) at a freezing rate of 4 LRC images. Range of fluorescent parameters is for determination of different light intensities that describe photochemical and non-photochemical efficiency of photosystem II (PSII).

Fig 4 Rapid changes in fluorescence parameters 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 (0.6 of the access light used in the LRC protocol. Chlorophyll fluorescence parameters were measured for 7 days following the NaCl treatment. Changes in non-photochemical quenching (NPQ) two days after salt stress treatment were 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 photosynthetic quantum yield (Fv/Fm) and was accompanied by a reduction of quantum yield of PSII photochemistry. (Average ± SD, 4-8 genotypes per condition).

Fig 5 Representative greenness index images of Col-0 and C24 Arabidopsis accessions for control and salt stress treated plants taken at the last day of measurement (21.03.2007). Day 7 after NaCl treatment. RGB/visible images were used quantified growth, color index and other morphological parameters in non-destructive manner dynamically during the onset of salinity to address shoot/vein independent phase of salinity stress (Brajková et al. 2009).

Fig 6 Schematics of light response curve (LRC) protocol used for determination of photosynthetic function in control and salt-stressed plants. LRs are used to quantify the rate of photosynthetic performance of different light irradiances and were broadly applied as a 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 (14 LRC to 1400 µmol m⁻² s⁻¹) at a freezing rate of 4 LRC images. Range of fluorescent parameters is for determination of different light intensities that describe photochemical and non-photochemical efficiency of photosystem II (PSII).

Fig 7 Schematics of light response curve (LRC) protocol used for determination of photosynthetic function in control and salt-stressed plants. LRs are used to quantify the rate of photosynthetic performance of different light irradiances and were broadly applied as a 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 (14 LRC to 1400 µmol m⁻² s⁻¹) at a freezing rate of 4 LRC images. Range of fluorescent parameters is for determination of different light intensities that describe photochemical and non-photochemical efficiency of photosystem II (PSII).