PlantScreen as a new tool for high throughput plant phenotyping

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INTRODUCTION

Drought and salinity in soils are currently one of major problems in agriculture with rapidly growing importance in last few years.

The use of advanced phenotyping that offers application of automated, high-throughput methods for characterisation of plant architecture and performance, has the potential for improvement of breeding process of different important crops.

Conveyor and robotic PlantScreen system developed in PSI s.r.o. is designed to serve as high throughput screening platform for plant phenotyping in various species from *Arabidopsis* to crop plants. PlantScreen is configured for non-destructive measurement and imaging technologies in automated, software operated system. PlantScreen consists of growth and acclimatisation chamber with automated weighting and watering system, and several modules designed for complex automated kinetic phenotyping including chlorophyll fluorescence imaging, thermal imaging, RGB imaging and hyperspectral imaging station.

We have used the PlantSreen system to study response of different *Arabidopsis* accessions to salt and drought stress conditions. We have optimized the screening conditions and the image processing analysis to obtain quantitative assesment of plant complex traits as growth, development and physiological status.

Chlorophyll fluorescence has long been regarded as sensitive indicator for water and salt stress (1), therefore we focused primarily on kinetic chlorophyll fluorescence measurements and assesment of morphological parametres in control and salt treated Arabidopsis plants.

We have optimised screening conditions for plants of different developmental stages and various salt concentrations and methods of applications. Set of markers derived from chlorophyl fluorescence kinetic measurements were identified as potential early response stress indicators.

Conveyor and Robotic **PlantScreen ™ Systems** for plant phenomics analysis can be optimized for numerous plant morphologies and structures from *Arabidopsis* to crop plants.

RESULTS

Growth conditions and stress treatment

✓ *Various ecotypes of* Arabidopsis thaliana (Col, Ler, Co) *were grown under long day conditions and 150 µE illumination in PhytoScope FS 360 chamber. After 25 days plants were moved into PlantScreen, where the same growth conditions were applied in the growth and acclimatisation chamber.*

✓ *Salt stress as 250mM NaCl watering solution was applied by automated watering and weighing system for 6 days in 12 hours intervals.*

✓*Automated kinetic phenotyping analysis was performed by using fluorescence imaging and RGB structural imaging station.*

✓ ***First visible phenotypical changes were observed after 4-5 days from initiation of salt stress treatment.***

Visible phenotype of 30-d-old plants 6 days after salt solution was applied.

✓ *LED lightning in the acclimatisation chamber*

✓ *Automated weighing and watering system*

RGB Imaging

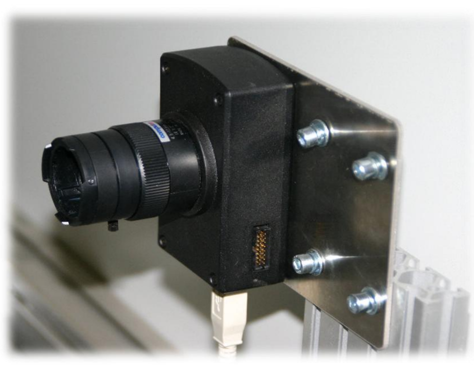
✓ *Morphological changes occuring throughout the growth and mediated in response to abiotic stress application were screened in time by using three RGB cameras installed in imaging station of PlantScreen System.*

✓ *Captured RGB imaging data were automatically saved in the PlantScreen database and image analysis was processed.*

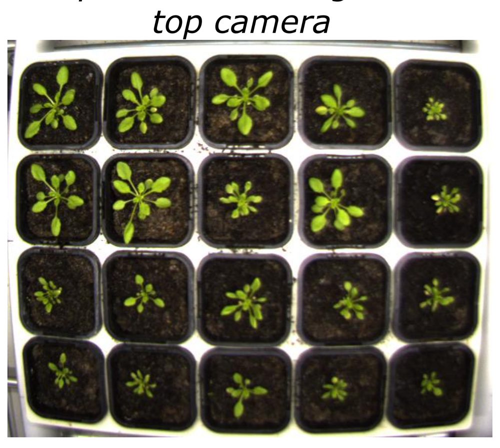
✓ ***Set of standardized morphological parametres such as area, roundness or compactness was assessed and used to discriminate between different phenotypes.***

✓ ***Set of morphological patterns was used to perform statistical analysis for morphological trait based grouping.***

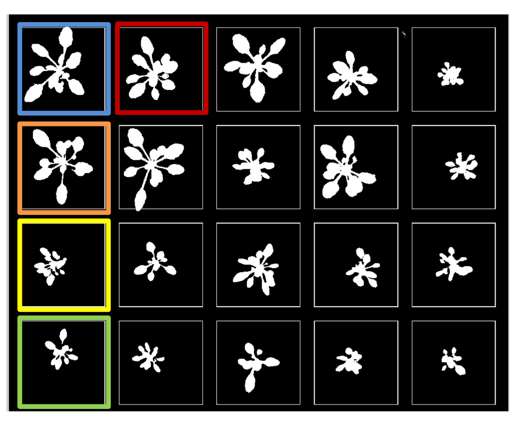
✓*RGB structural imaging*



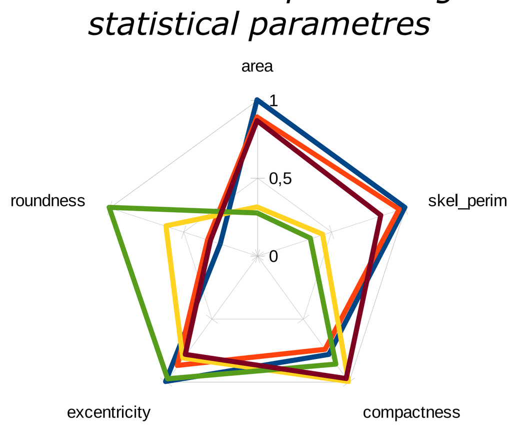
Captured RGB image from top camera



Automated image processing



Calculation and processing of statistical parametres



25-d-old Col and Co Arabidopsis plants prior stress treatment.

Fluorescence Imaging

✓ *Automated kinetic chlorophyll fluorescence imaging (2) was performed prior the initiation of salt stress treatment and during the application of salt solution in defined time intervals.*

✓ *Queching analysis that allows to measure and calculate complex set of photosynthetic parametres was used to assess photosynthetic dynamics in the intact Col and Ler plants.*

✓ *Analysed measured parametres were : Fv, Fm, Fv, Fo', Fm', Fm', Ft*

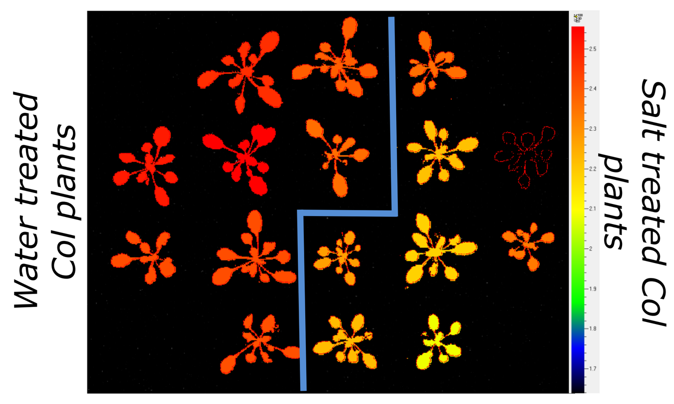
Set of calculated parametres: Fv/Fm, Fv'/Fm', Phi_{PSII} , NPQ, qN, qP, Rfd

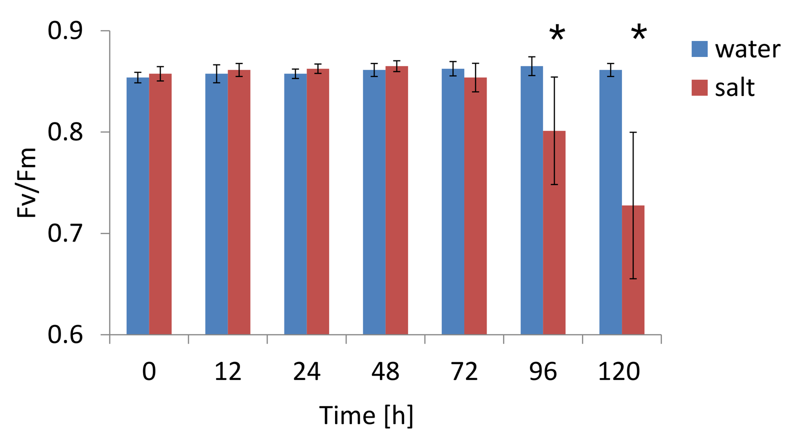
✓ ***Complex analysis of photosynthetic parametres allowed to discriminate early response stress markers such as NPQ and Rfd.***

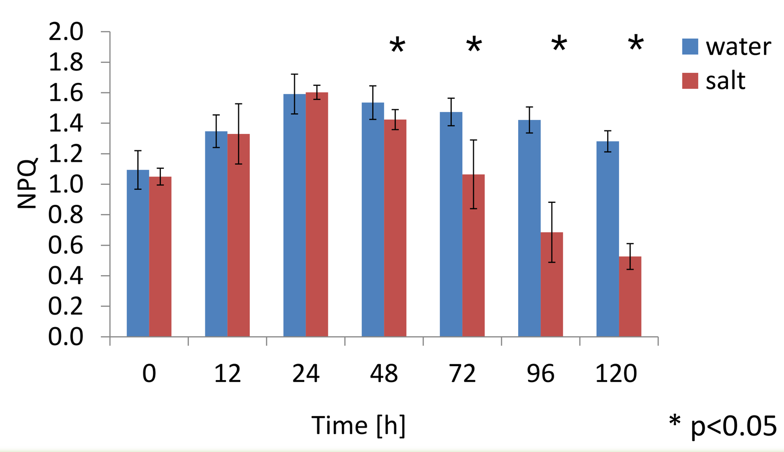
✓***Statistically significant differences between treated and non-treated Col plants were observed already 48h after salt stress application.***

✓ *Fluorescence imaging station*

✓ *Automated discrimination based on RFD (plant fitness) parametr*







CONCLUSIONS & OUTLOOK

✓ ***Based on chlorophyll fluorescence kinetic measurements set of early response markers was identified that might allow to discriminate between stress resistant and stress sensitive plants already within first 48h upon stress treatment in Arabidopsis thaliana .***

✓***Our results suggest that parametres such as NPQ and Rfd seem to reflect early stress responses faster than Fv/Fm parametr.***

➢***Optimisation of the screening conditions and data processing for the automated phenotyping of crop species.***

➢***Include thermal imaging and hyperspectral imaging station for complex phenotypic analysis.***

REFERENCES

1. Mishra KB, Iannacone R, Petrozza A, Mishra A, Armentano N, La Vecchia G, Trtílek M, Cellini F, Nedbal L. (2012): Engineered drought tolerance in tomato plants is reflected in chlorophyll fluorescence emission. Plant Science 182, 79-86.
2. Nedbal L., Soukupova J., Kaftan D., Whitmarsh J., Trtilek M. (2000): Kinetic imaging of chlorophyll fluorescence using modulated light. Photosynthesis Research 66, pp. 3-12.