Revealing Plant Responses to Combined Abiotic Stresses Using Image-based Phenotyping

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Introduction

Investigating the underlying mechanisms of plants under combined abiotic stresses to identify traits of interest for climate-resilient crops is highly demanded. This study, conducted within the framework of the ADAPT project, aimed to investigate the dynamic responses of potato plants- as one of the most important food crop- to single and combined abiotic stresses using high throughput phenotyping protocol and integrating to other omics level.

kinetic chlorophyll fluorescence imaging under light adaptation conditions was selected to non-invasively assess photosynthetic efficiency in response to stress at the canopy level- which is considered as one of rapidly screening tool. Additional imaging sensors were combined for an integrative investigation of morphological and physiological responses.

Methods

- This study was conducted at PSI Plant Phenotyping Research Center (PSI, Drásov, Czech Republic) equipped with state-of-art infrastructure for plant cultivation and automated high-throughput phenotyping platform under controlled conditions.
- Two potato cultivars (Solanum tuberosum cv.) were selected, Désirée & Lady Rosetta, the latter being more stress-tolerant.
- As shown schematically in Figure 1. the stress treatments were applied as the following: Heat (H)– was induced at 30/28°C day/night for 2 weeks; Drought (D)– was induced for 1 week; Waterlogging (W)–was induced for 5 days to avoid detrimental impact followed by recovery; Heat + Drought + Waterlogging stress (HDW)– started with 1 week of inducing heat, then progressive drought for another week followed by 1 day of waterlogging.
- Three water regimes were applied: 60% filed capacity (FC) in C and H conditions, 30% FC in D conditions, and 130% FC in W conditions.
- The phenotyping was conducted daily on a total of 50 plants with final yield assessment at the last day of the phenotyping. This phenotyping was integrated with other multiple omics analysis therefore additional plants were harvested. Successive harvesting of the 2nd, 3rd and 4th youngest fully developed leaves for follow-up transcriptomics, metabolomics, hormonomics, and proteomics analyses.

Protocol overview

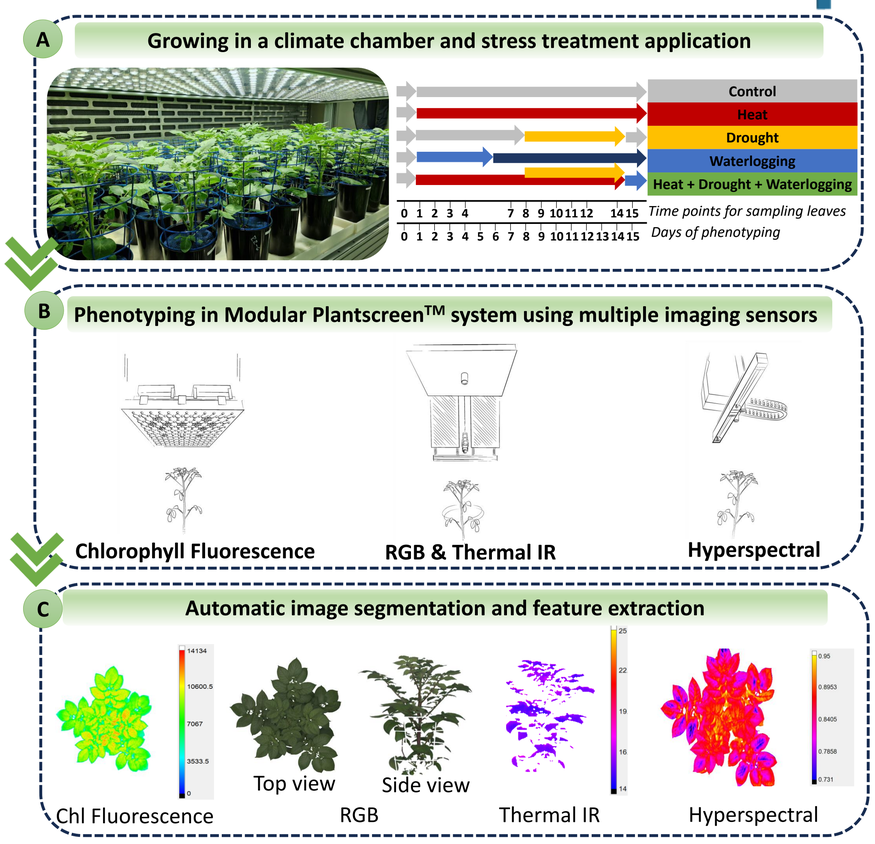

Figure 1. Schematic overview of the applied phenotyping protocol and stress duration

Results

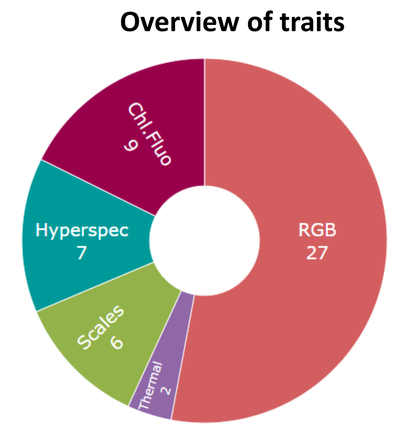

Figure 2. Wide range of morphological, physiological and leaf spectral-related traits were extracted from the multiple imaging sensor. In total 51 traits were extracted over 16 timepoints.

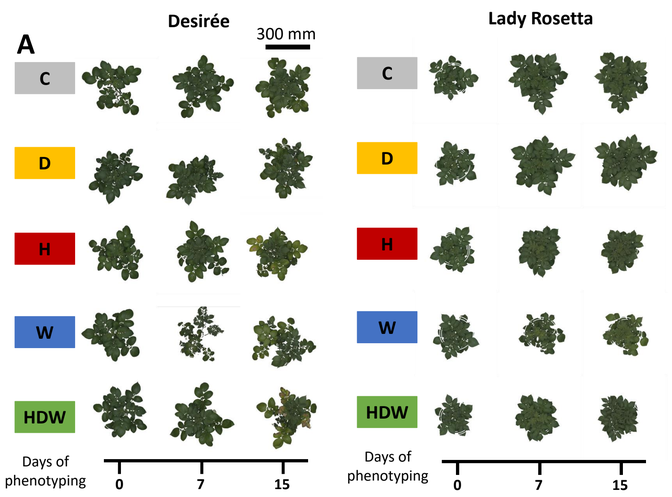

Figure 3. Dynamic growth response from RGB imaging. (A) RGB top view images in both cultivars. (B) Plant volume at 3 time points. (i) 0-5 DOP, indicated W stress response and early H, (ii) 6-10 DOP, reflected the early D and HD and (iii) 11-15 DOP, showed the late heat and drought responses and HDW.

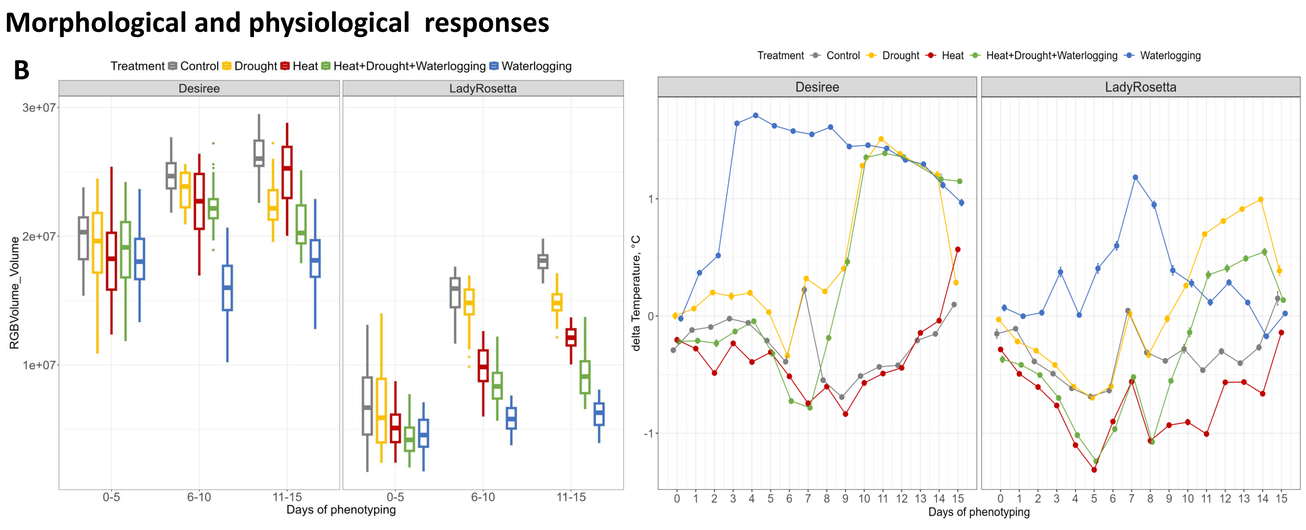

Figure 4. Stomatal regulation from thermal IR imaging, delta temperature –normalized canopy temperature to ambient temperature.

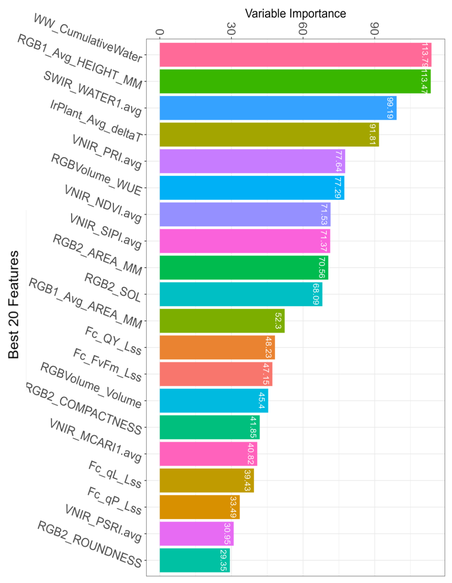

Figure 7. Variable importance of traits from Boruta feature selection to find which relevant features are more essential.

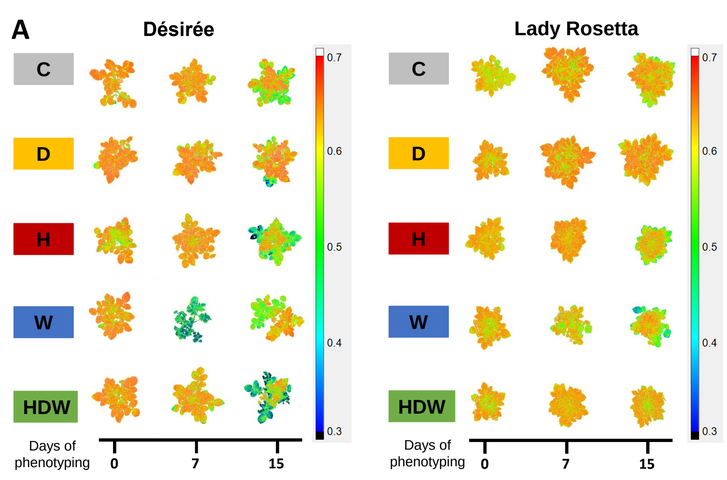

Figure 5. Photosynthetic efficiency from chlorophyll Fluorescence imaging. (A) Pixel-by-pixel false colour images of PSII operating efficiency in light steady state. (B) Maximum quantum efficiency of PSII at light steady state (Fv/Fm_Lss) during days of phenotyping.

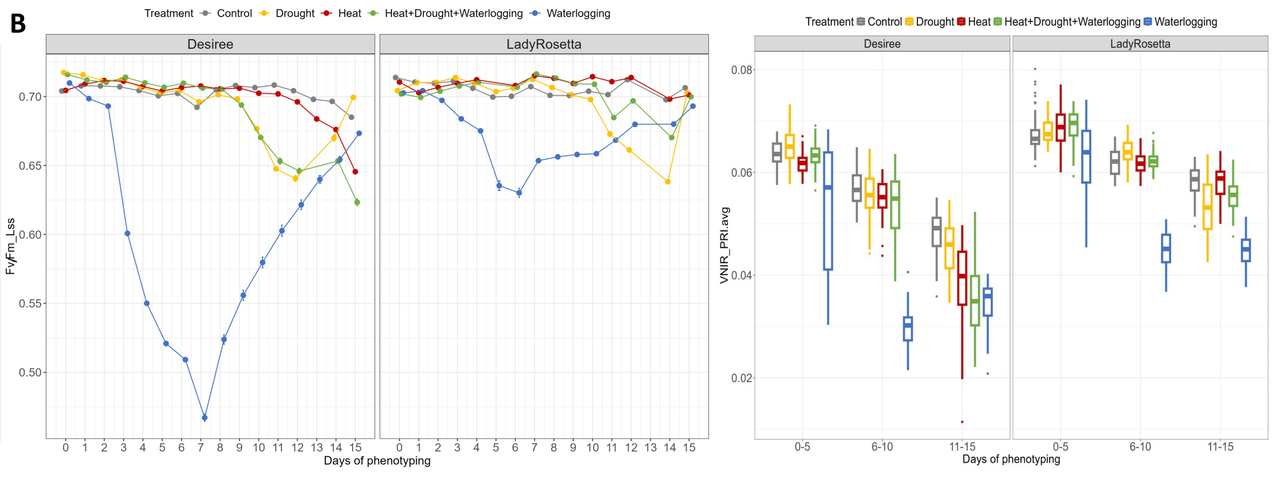

Figure 6. Photochemical Reflectance Index (PRI) to assess light use efficiency from visible near-infrared (VNIR) hyperspectral imaging.

Main findings

The results showed that both cultivars exhibited morphological and physiological changes in response to W, with more pronounced stress tolerance differences observed during the late phase of all stresses.

- **Growth dynamics**: Desiree (DE) had a higher plant volume than Lady Rosetta (LR) (Fig. 3). DE showed a pronounced reduction in plant volume, especially under W, combined HD, and late H stresses. Whereas, LR plant volume decreased under H, being lower under HDW stress.
- **Leaf cooling efficiency**: LR showed better cooling mechanisms under different treatments compared to DE as observed in lower delta T and cooler canopy temperature that had a positive impact in mitigating heat stress (Fig. 4). However, LR was more susceptible to single D than the combined HDW stress.
- **Photosynthetic performance**: Both cultivars experienced a reduction in Fv/Fm_Lss during the late phase of all stresses, with DE showing more pronounced reductions (Fig. 5). That was also reflected in PRI reduction indicating the lower efficiency in utilizing absorbed light in photosynthesis (Fig. 6).
- **Susceptibility to stress**: DE was more susceptible to W and late heat and HDW stresses as indicated by a significant reduction in growth rate, quantum yield, photosystem efficiency, physiological responses related to stomatal closure, and an increase in canopy temperature.
- **For integrative visualization**: Cumulative water content showed high variable importance (Fig. 7). When combining all traits together from multiple imaging sensors, PCA mainly separated the two cultivars. DE plants stressed under W, D, HD, and HDW, and LR plants stressed under D, clustered together (Fig. 8).

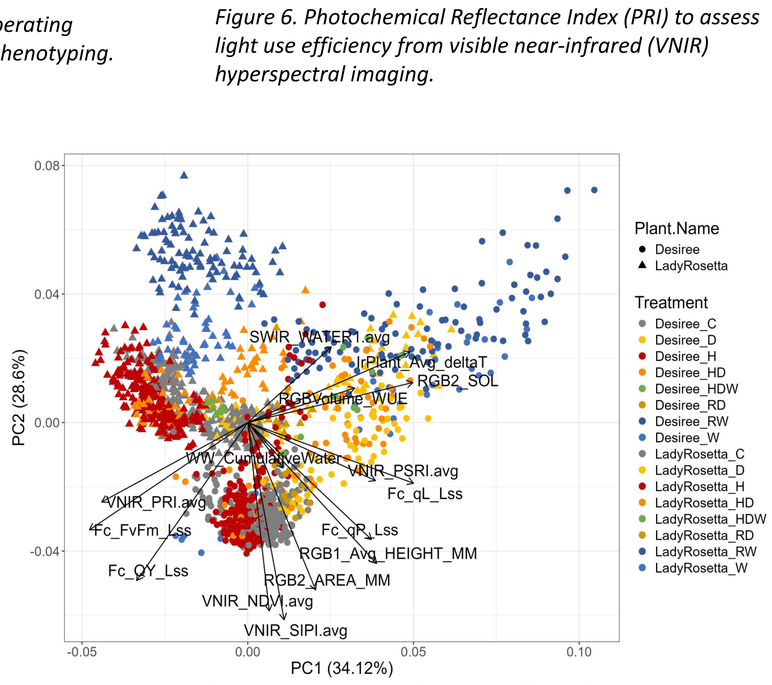

Figure 8. Principal component analysis (PCA) is performed on selected features among all days of phenotyping.

Conclusion

- The applied phenotyping protocol based on using a combination of multiple imaging sensors is a valuable tool for revealing new insights into understanding plant response in coping with climate change.
- Overall plant performance and photosynthetic efficiency were severely affected by waterlogging reflecting the detrimental effect of this stress on potato plants.
- Different responses to stress in tolerant and susceptible cultivars were determined under different stress and detection of early and late responses to stresses were identified.

Acknowledgment

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