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

Lamis Abdelhakim[1], Barbora Pleskačová[1], Klára Panzarová[1], Maja Zagorščak[2] and Markus Teige[3]

PSI (Photon Systems Instruments), Spol. S R.O., Czech Republic 1
National Institute of Biology, Ljubljana, Slovenia 2
University of Vienna, Austria 3

Introduction

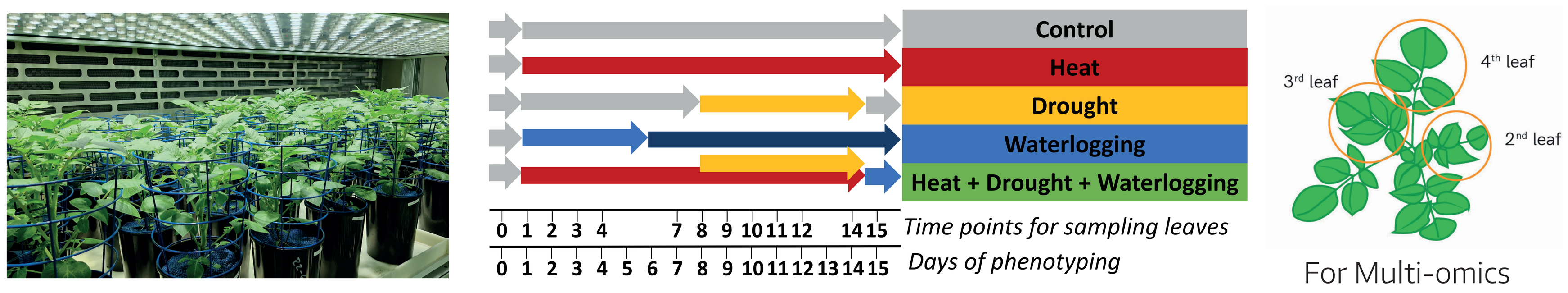
Understanding the mechanisms by which plants acclimate to combined abiotic stresses is crucial for developing climate-resilient crops. In frame of the ADAPT project, we employed advanced automated phenotyping tools to investigate the stress adaptation mechanisms of two contrasting potato cultivars, **"Désirée"** and **"Lady Rosetta"**. We provide detailed quantitative insights into how each cultivar responds to multiple abiotic stress factors. Our findings reveal significant differences in stress tolerance mechanisms between the two cultivars, highlighting specific traits that could be targeted in breeding programs to enhance crop resilience in variable climates. Potato stress resilience to single and combined drought, heat, and waterlogging stress was assessed by using PlantScreenTM phenotyping platform (PSI Research Center, Drásov, Czech Republic) integrating both physiological phenotyping tools (kinetic chlorophyll fluorescence imaging, thermal imaging) and structural phenotyping (RGB, hyperspectral imaging) in semi-controlled greenhouse conditions. A powerful data analysis pipeline was established to reveal specific signatures during potato growth and tuber formation.

Methods

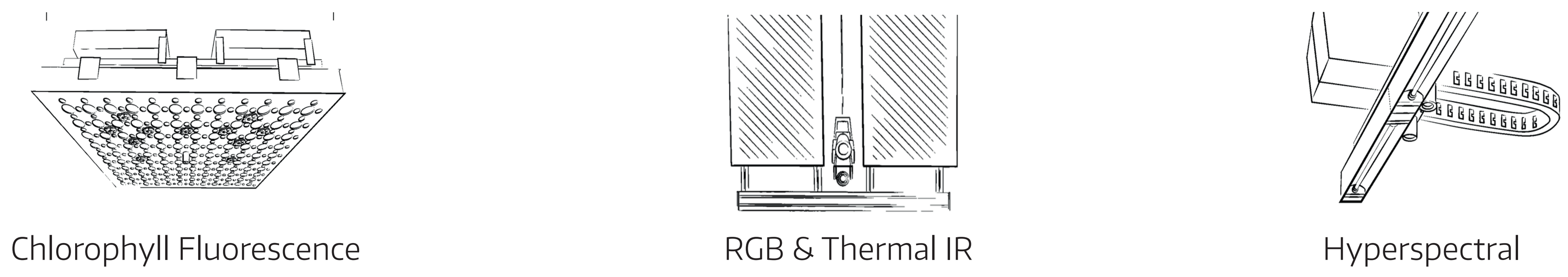
- Two potato cultivars *(Solanum tuberosum cv.)* **Désirée (DE) & Lady Rosetta (LR)** were selected, the latter being more stress-tolerant.
- **Fig.1A:** stress treatments were applied as the following: **Heat (H)** – was induced at 30/28 °C day/night for 2 weeks; **Drought (D)** – was induced for 1 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 % field capacity (FC) in C and H conditions, 30 % FC in D conditions, and 130 % FC in W conditions.
- The phenotyping was conducted daily on 50 plants with final yield assessment on the last day of the phenotyping. Sample harvest of the 2nd, 3rd and 4th youngest fully developed leaves was done for multiple omics analyses **(transcriptomics, metabolomics, hormonomics, and proteomics)** and was linked with phenomics analysis.

Protocol overview

A Growing in a climate chamber and stress treatment application



B Phenotyping in Modular Plantscreen™ system using multiple imaging sensors



C Automatic image segmentation and feature extraction

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

Results

Growth dynamics: **Désirée (DE)** had a higher plant volume than **Lady Rosetta (LR)** (Fig. 2). When stress was induced, **DE** showed a pronounced reduction in plant volume, especially under W and even after recovery, combined HDW. Whereas, **LR** showed a stagnation in plant volume under W and decreased under Heat treatments, being lower under HDW stress.

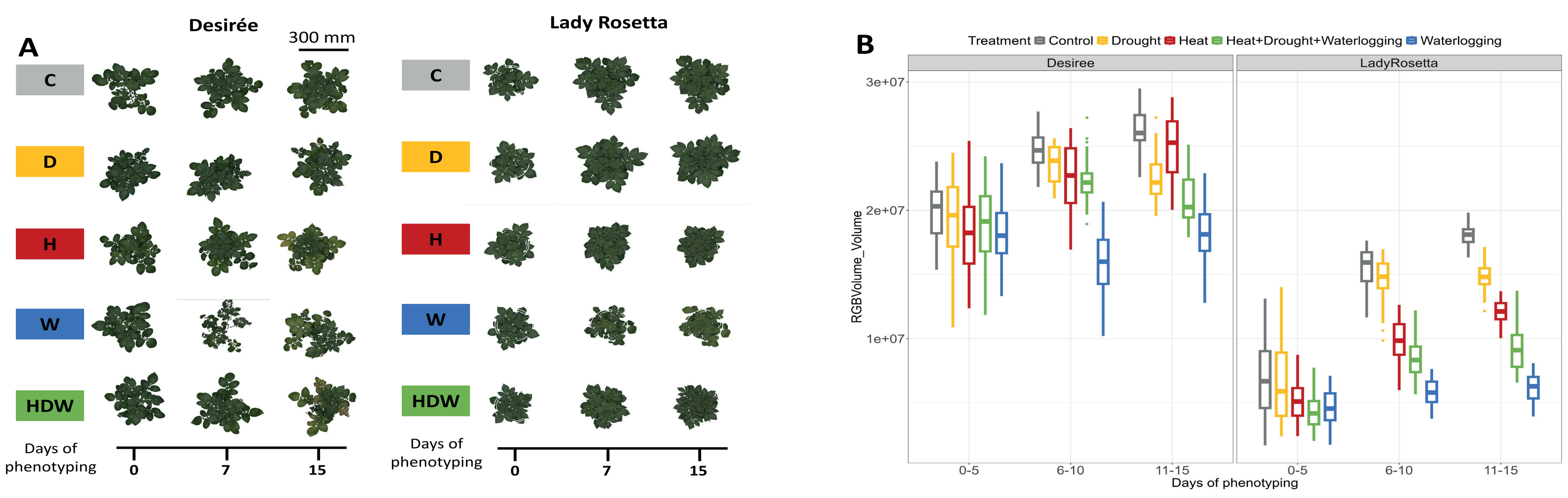


Figure 2. (A) RGB top view images in both cultivars. (B) Plant volume from RGB top and side view imaging at 3 time points. (i) 0-5 DOP, indicated W stress response and early H, (ii) 6-10 DOP, reflected the early D and HD and (iii) 11-15 DOP, showed the late heat and drought responses and HDW.

Leaf cooling efficiency: **LR** showed better cooling mechanisms under different treatments compared to **DE** as shown by lower delta T and cooler canopy temperature that had a positive impact in mitigating heat stress (Fig. 3). In spite, **LR** was more susceptible to single D than the combined HDW stress, a fast recovery was observed in all treatments.

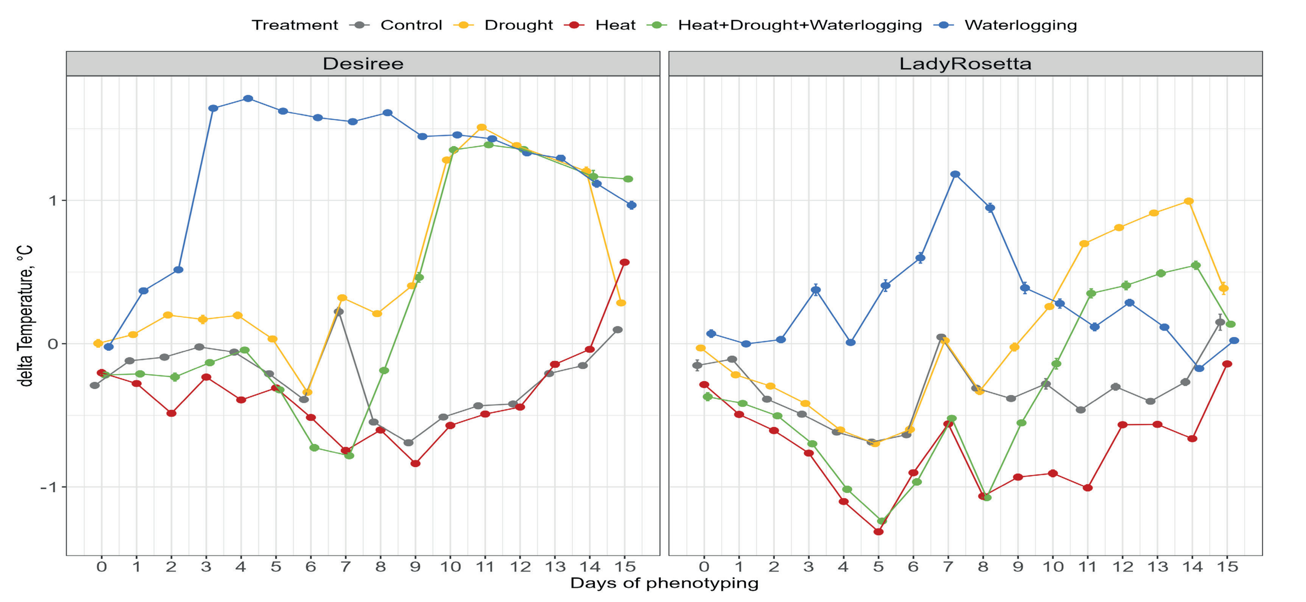


Figure 3. Stomatal regulation was assessed by thermal IR imaging. DeltaTemp is normalized canopy temperature to ambient temperature. Data represent mean and standard error of mean (n = 10).

Photosynthetic performance: **DE** was more susceptible to W and late H and HDW stress as indicated by a significant reduction in photosynthetic efficiency (Fv/Fm_Lss) (Fig. 4). Similar trend was reflected in PRI reduction as well indicating the lower efficiency in utilizing absorbed light in photosynthesis (Fig. 5).

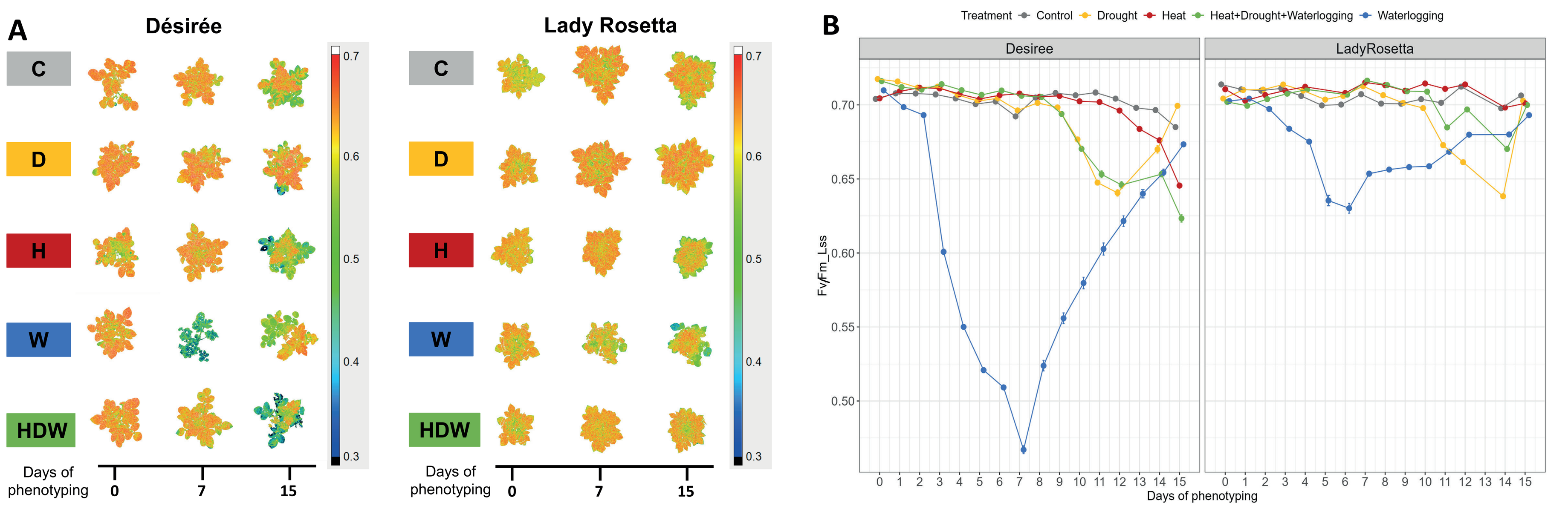


Figure 4. (A) Pixel-by-pixel false colour images of PSII operating efficiency in light steady state from chlorophyll fluorescence imaging. (B) Maximum quantum efficiency of PSII at light steady state (Fv/Fm_Lss) during days of phenotyping. Data represent mean and standard error of mean (n = 10).

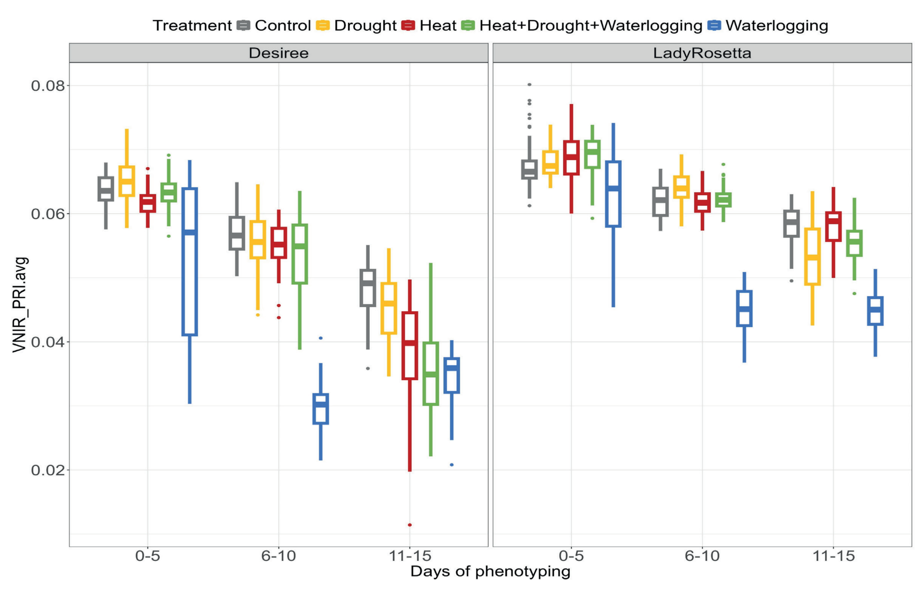


Figure 5. Photochemical Reflectance Index (PRI = R531-R570 / R531+R570) to assess light use efficiency from visible near-infrared (VNIR) hyperspectral imaging.

Conclusion

We show 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 where **DE** was more susceptible to stress.

- Integrative phenotyping combining **multiple imaging sensors** is a valuable tool for revealing new insights into understanding plant resilience.
- **Stress acclimation** mechanisms to early and late stress responses were specifically characterized for the cultivars and discriminated by specific traits.
- Overall plant performance and **photosynthetic efficiency** were severely affected by **waterlogging** reflecting the detrimental effect of this stress on potato plants and resulting in yield reduction.
- Detailed insights into stress adaptation mechanisms in potato plants will be underlined by integrating all omics data into the **knowledge network pipeline** (Zagorščak et al., 2023).

Integrative visualization: Morphological, physiological, and leaf spectral-related traits were extracted from multiple imaging sensors (Fig. 6). The water index (WATER1) (SWIR) and deltaTemp (Thermal IR) showed high variable importance (Fig. 7). When combining **top 20 traits,** PCA mainly separated the two cultivars. **DE** plants stressed under W, D, HD, and HDW, and **LR** plants stressed under D, clustered together and strongly correlated with 4 traits: water index, deltaTemp, water use efficiency (WUE), and solidity (SOL) (Fig. 8).

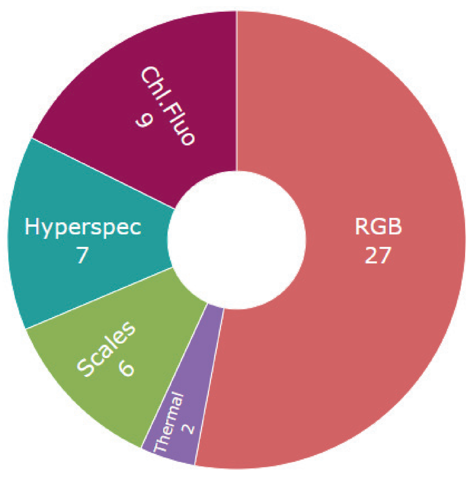


Figure 6. In total 51 traits were extracted over 16 time points.

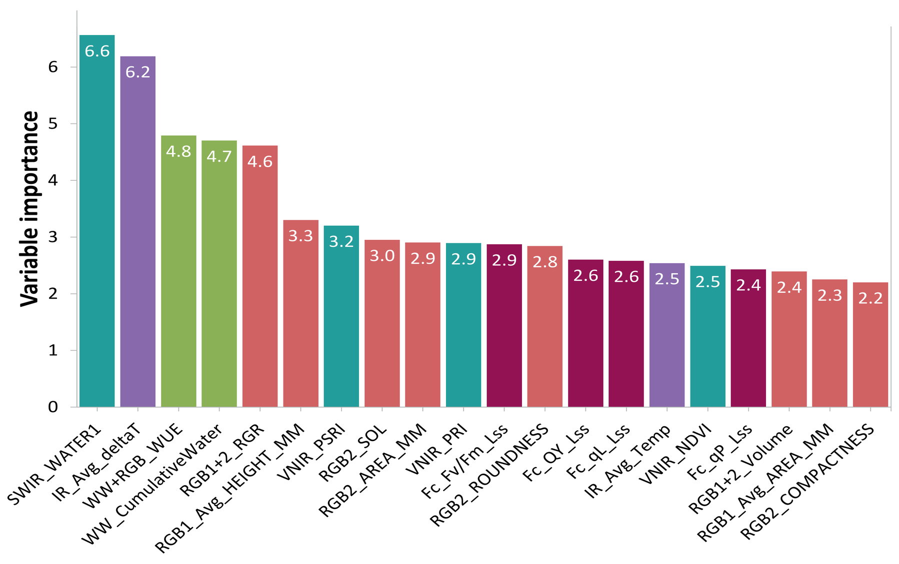


Figure 7. Variable importance of traits using random forest to identify and select top 20 key features.

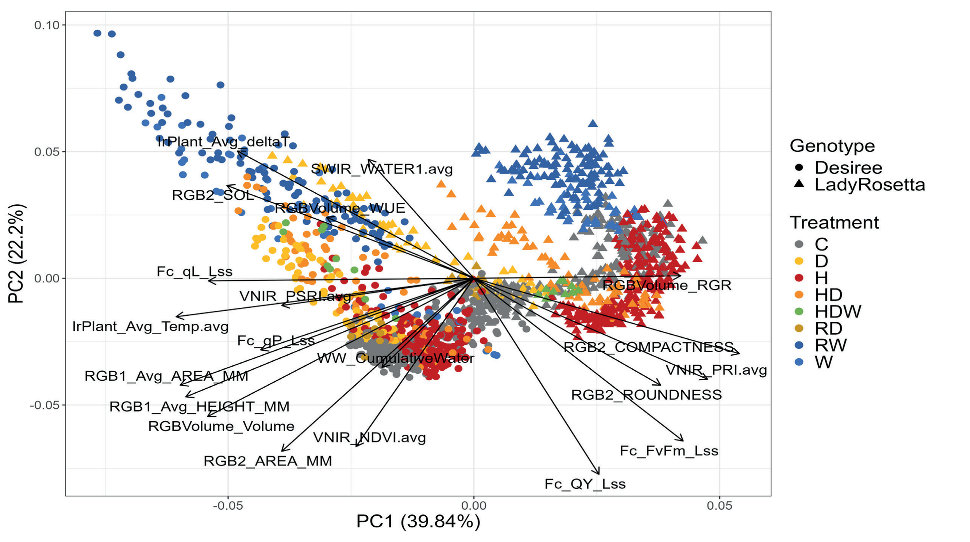


Figure 8. Principal component analysis (PCA) is performed on selected features among all days of phenotyping. RD-refers to recovery from Drought; RW – recovery from Waterlogging.

Acknowledgment

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References

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