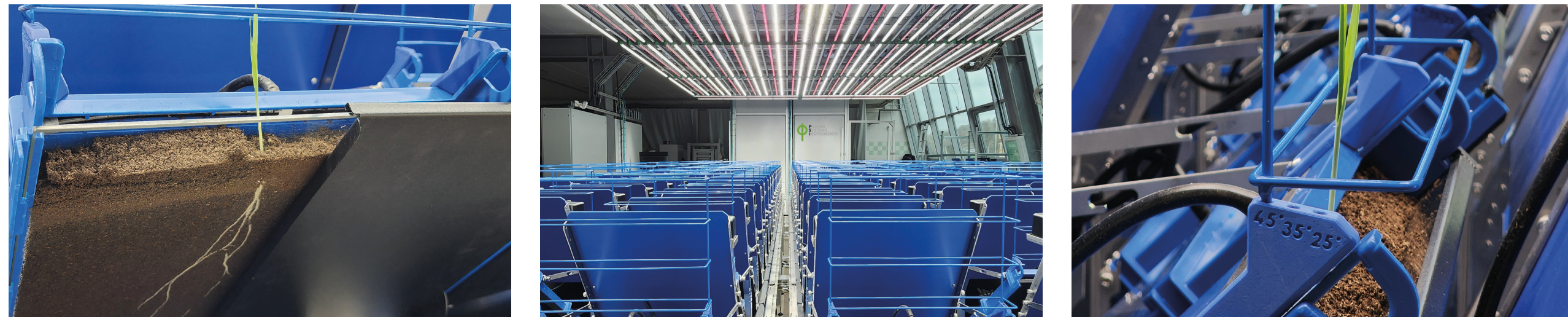


# Belowground Dynamics Revealed Root-to-Shoot Stress Response Dynamics: A Case Study of Barley Drought Resilience

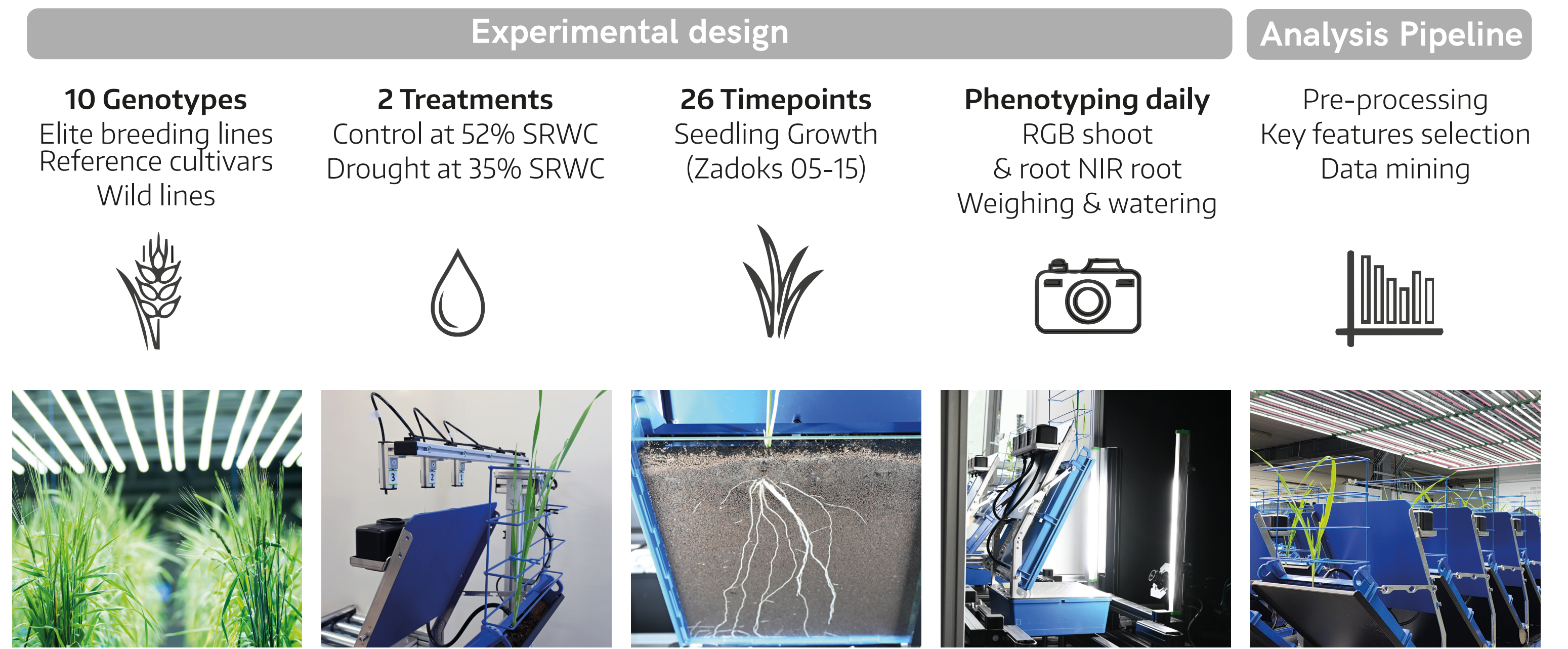
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## Introduction

High-throughput phenotyping has advanced shoot-level studies, but root phenotyping remains limited due to destructive and time-consuming methods. We present applications of the **PlantScreen™ Root System** (PSI Research Center, Drásov, Czech Republic), an automated, non-invasive platform for parallel monitoring of root and shoot growth under different moisture regimes. Within the **BarleyMicroBreed project**, aiming to provide crop breeders with new strategies for the development of drought-resilient cultivars genetically fit to harness the benefits of root microbiomes. Ten barley genotypes were selected for phenotyping based on previous field trials. The root and shoot morphological traits were assessed at the tillering stage under moderate drought stress. Using RGB imaging for root and shoot growth dynamics and near-infrared imaging for assessing moisture content in the soil profile. An integrative data analysis pipeline was established and used to reveal specific signatures during growth.



## Protocol overview



## Results

### NIR-Based Moisture Index

Soil moisture index was monitored using NIR imaging (reflectance 940 and 1450 nm) in control and drought treatments defined by different initial soil relative water content (SRWC). Rhizobox weight was monitored by automatic weighing and watering. Control was maintained at a reference weight, while drought was not rewatered.

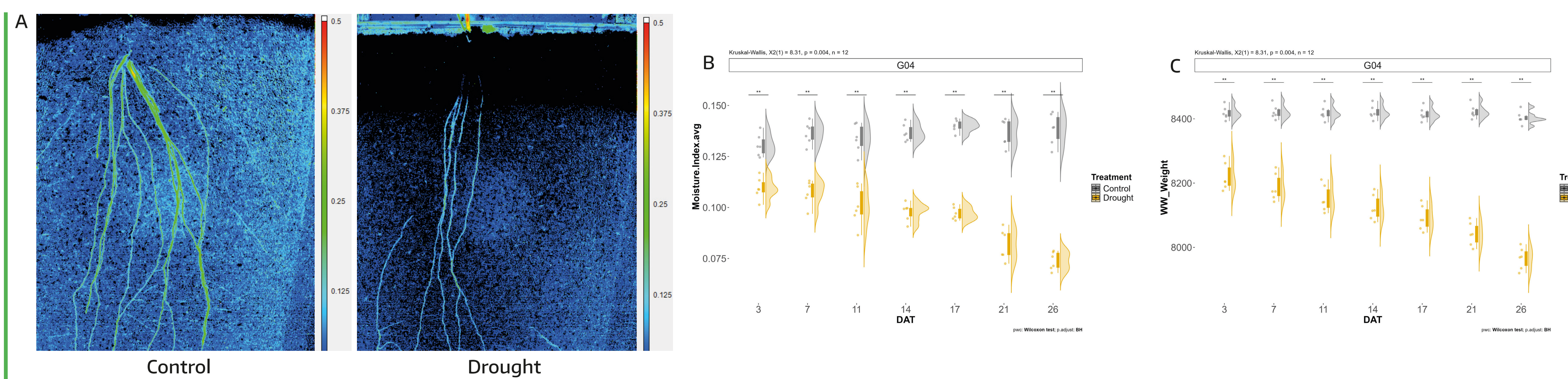


Figure 1 A) False-color Segmented NIR image for control and drought soil profile. B) Water index over days after transplant (DAT). C) Rhizobox weight monitored daily.

### Growth dynamics

Clear discrimination between treatments was observed in both shoot and root areas. In control plants, secondary roots emerging from the basal region showed dense root-hair development, whereas drought-stressed plants showed reduced root development, reflecting responses to differing soil moisture gradient conditions.

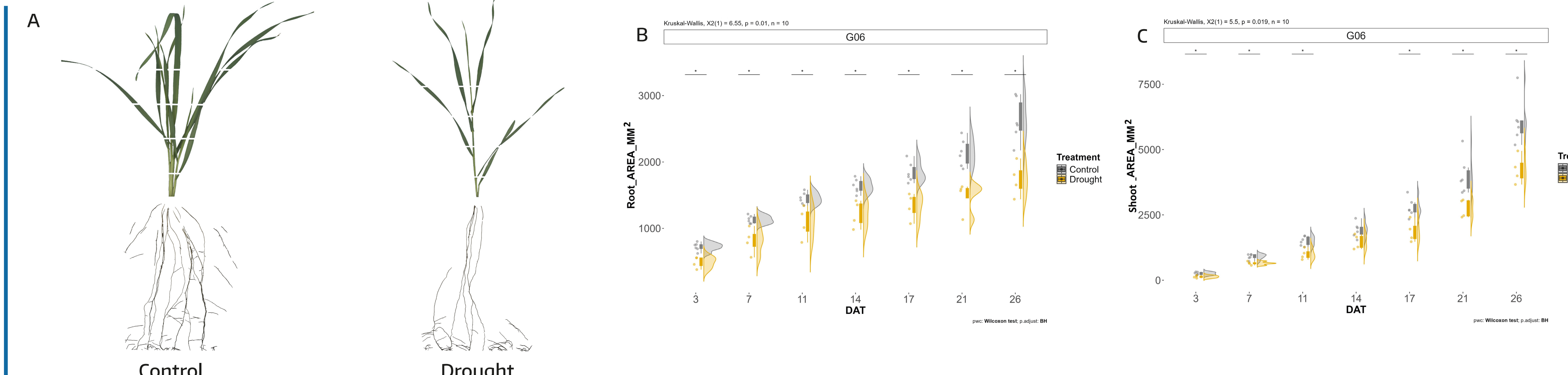


Figure 2 A) Parallel RGB segmentation of above- and below-ground structures. B-C) Temporal changes in root and shoot area measured using RGB over days after transplant (DAT).

### For integrative visualization

Root-related traits showed high importance in a random forest model, with root height (depth) emerging as a key trait for discriminating between treatments, followed by root distribution median. PCA separated the treatments, with the Root: Shoot ratio strongly associated with early drought response, reflecting faster root growth.

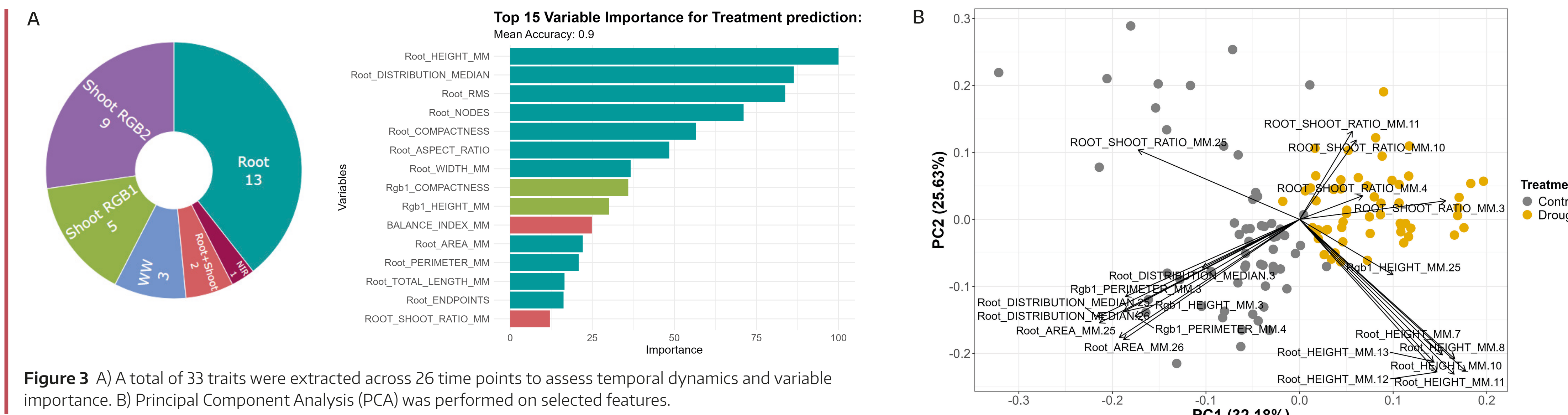


Figure 3 A) A total of 33 traits were extracted across 26 time points to assess temporal dynamics and variable importance. B) Principal Component Analysis (PCA) was performed on selected features.

### Resource allocation

Genotypes showed differences in root architecture and root density distribution, reflecting different strategies of resource acquisition. By combining both root and shoot area, some showed greater shoot investment relative to root growth, reflected in a higher balance index in the 3rd phenotyping week, indicating shifts in root to shoot allocation.

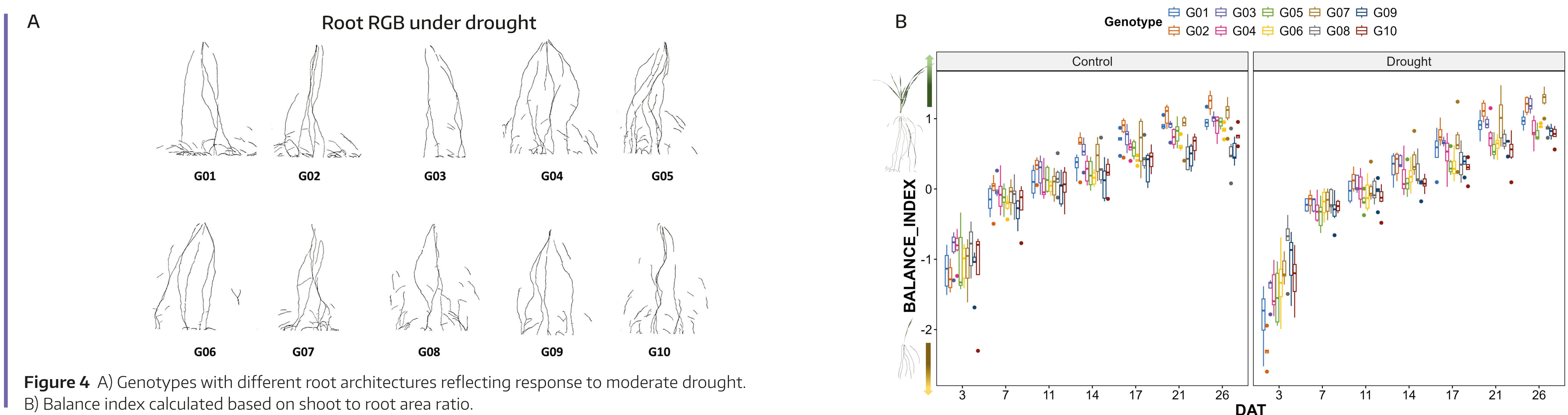


Figure 4 A) Genotypes with different root architectures reflecting response to moderate drought. B) Balance index calculated based on shoot to root area ratio.

### Conclusion

Parallel monitoring of root and shoot responses enabled clear discrimination between treatments and revealed genotype-specific drought responses, providing an integrated view of resource allocation. The results highlight the importance of root-related traits in early-stage drought responses and support the identification of genotypes with root-shoot allocation patterns associated with stress adaptation.



### Acknowledgment

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