Investigating the Relationship between the Cell Wall Integrity Pathway and Unfolded Protein Response in *Arabidopsis thaliana*

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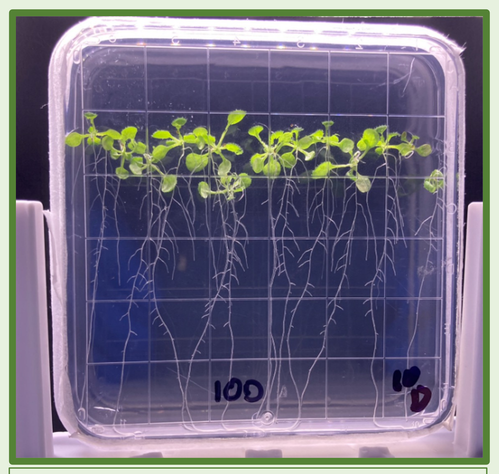
Introduction

- Understanding mechanisms of plant adaptation to spaceflight is critical as spaceflight-grown plants can balance space diets and ensure mission health.
- As crops grown in space exhibit alterations in gene expression related to cell wall modification, omics approaches to *Arabidopsis thaliana's* cell wall integrity (CWI) can help better understand plant adaptation to spaceflight. (Johnson et al., 2019; Kruse et al., 2020; Barker et. al., 2023).
- However, while there are transcriptomic studies on the relevance of the unfolded protein response (UPR, a conserved signaling cascade that responds to spaceflight stresses), the relationship between CWI and UPR is understudied (Angelos, et al., 2021).
- The goal of this study is to use a multi-omics approach to arrive at an evidence-based conclusion about the relationship between CWI and UPR.



Arabidopsis thaliana: model plant of space experiments
(Photo Credit: NASA)

Reanalysis of GLDS-321

Figure 1a: bZIP28 Mutant

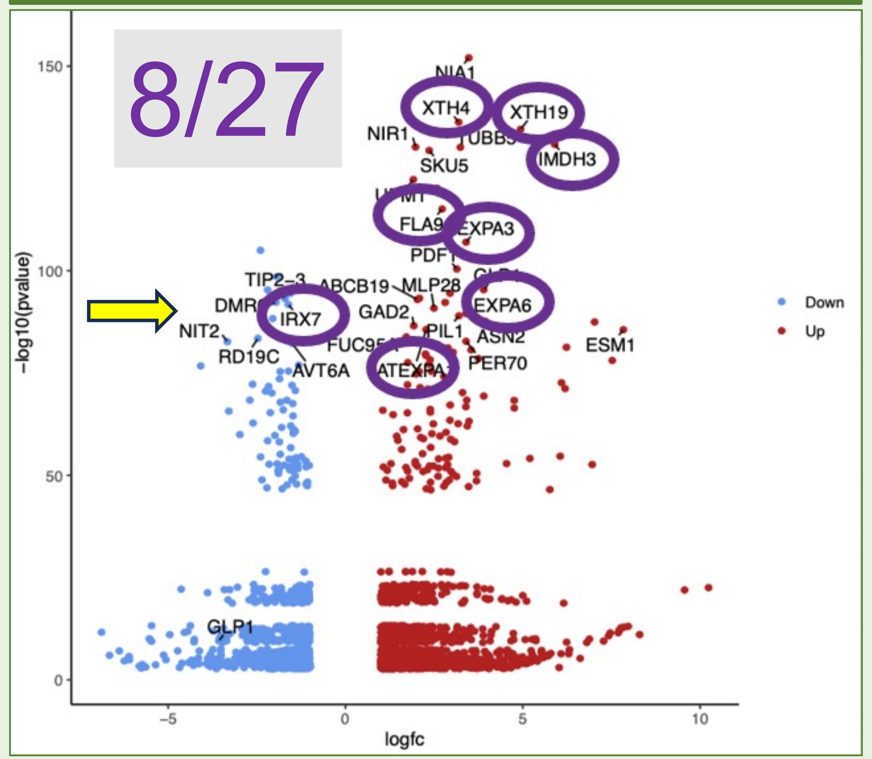
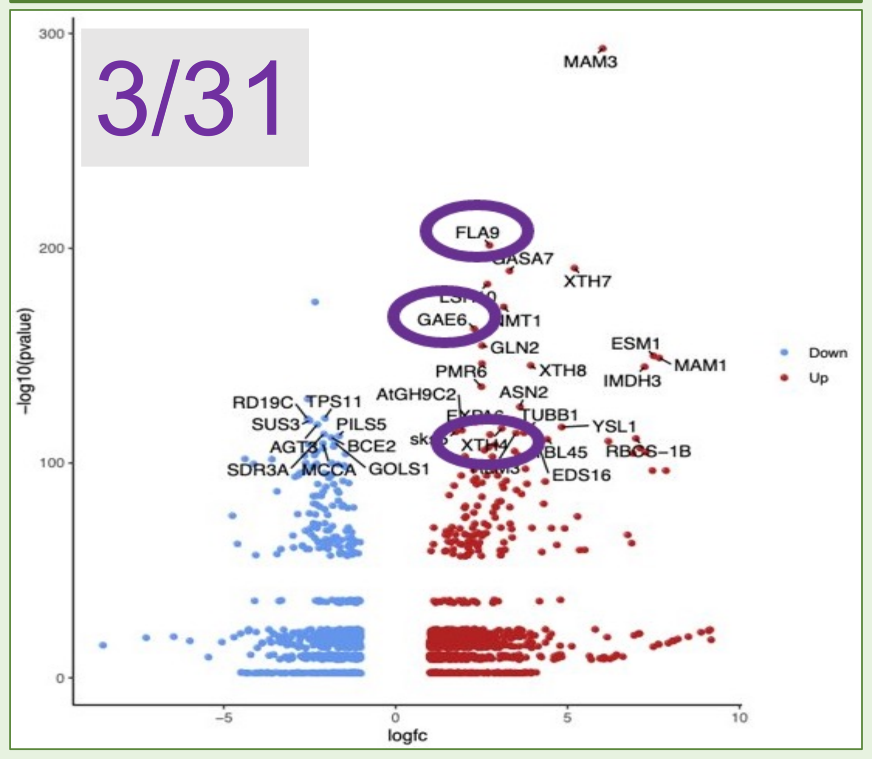


Figure 1b: Wild Type



- GeneLab Dataset 321 (GLDS-321): *Relevance of Unfolded Protein Response to Spaceflight-Induced Transcriptional Reprogramming in Arabidopsis* (PI: F. Brandizzi, NASA Open Science Data Repository).
- **Figure 1: The impact of spaceflight on gene expression in *A. thaliana*.** The *DESeq2* tool was used on *UseGalaxy.org* platform to determine the most significant and differentially expressed genes in the bZIP28 mutant (silenced bZIP28 gene*) and wide type (WT) (*p-value< 0.05; LogFC< 1.0*). Volcano plots indicate the relative expression of genes in spaceflight compared to ground control in the bZIP28 mutant **(Figure 1a)** and WT control **(Figure 1b)**. (*bZIP28: a key transcription factor in UPR)
  - In the bZIP28 mutant, 8 of the 27 significant genes were related to cell wall structure, including **IRX7**.
  - In WT, 3 of the 31 significant genes were related to cell wall structure.

Figure 2

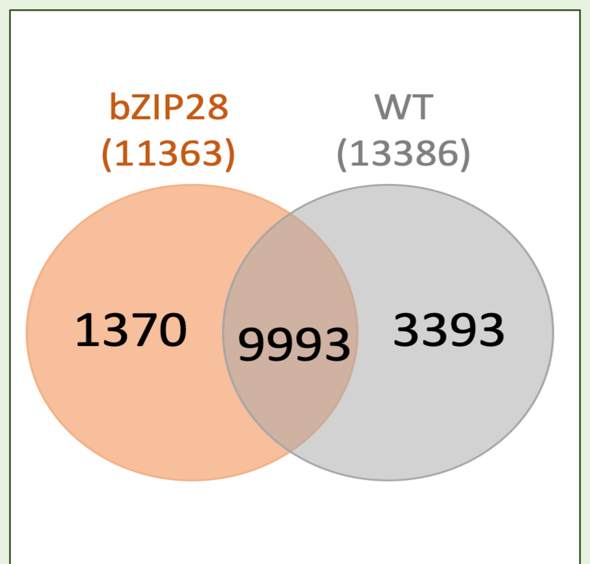


Figure 3a: bZIP28 Mutant

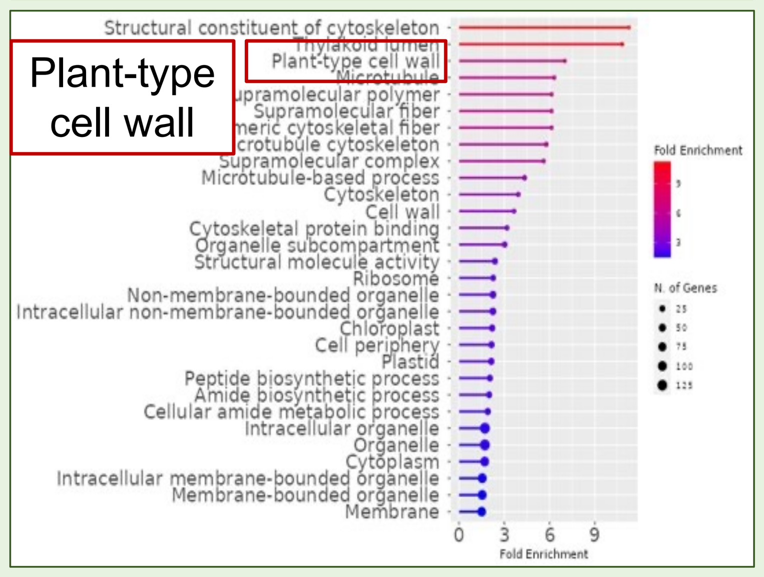
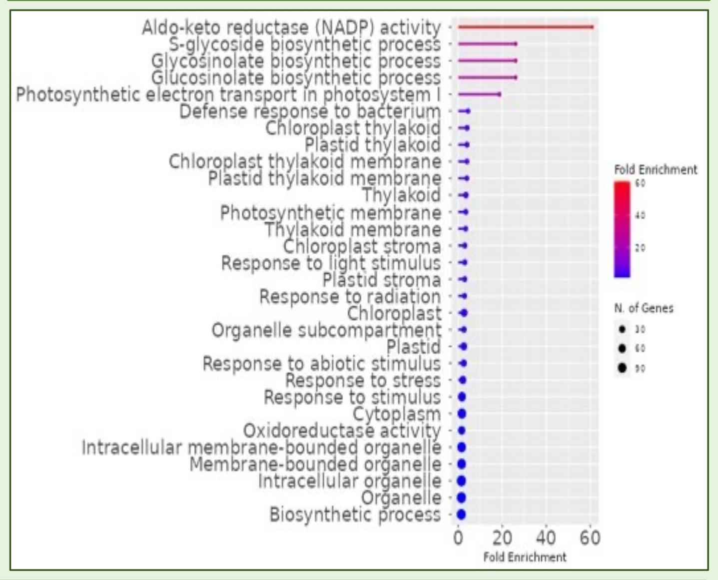


Figure 3b: Wild Type



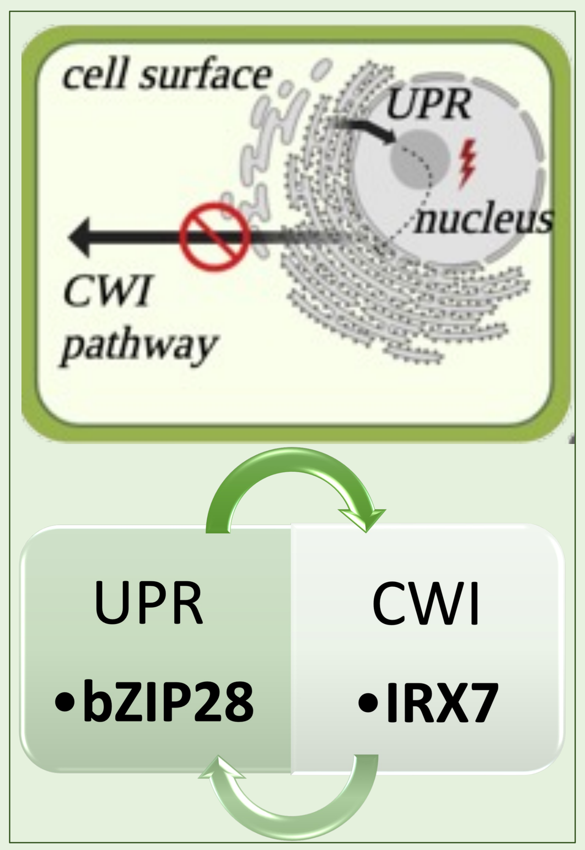
- **Figure 2: Shared gene expression changes in bZIP28 mutant and WT *A. thaliana*.** Using a Venn Diagram to compare the bZIP28 mutant and WT genes of *A. thaliana*.
- **Figure 3: The impact of spaceflight on CWI pathways in *A. thaliana*.** Lollipop plots highlight the most enriched pathways.
  - **(3a):** One of the most recurring pathways labeled as significant in bZIP28 mutant was cell-wall-related pathways (red square). This implies that bZIP28 deletion impacts the cell wall pathways.
  - **(3b):** In WT, no pathways related to the cell wall were detected in the enriched pathways, which highlights the impact of bZIP28 deletion on the cell wall pathways.

Hypothesis

Gene IRX7 (Irregular Xylem Gene7, oversees secondary cell wall biogenesis) affects the intracellular communication between the CWI pathway and UPR.

Proposed Experimental Design

Figure 4



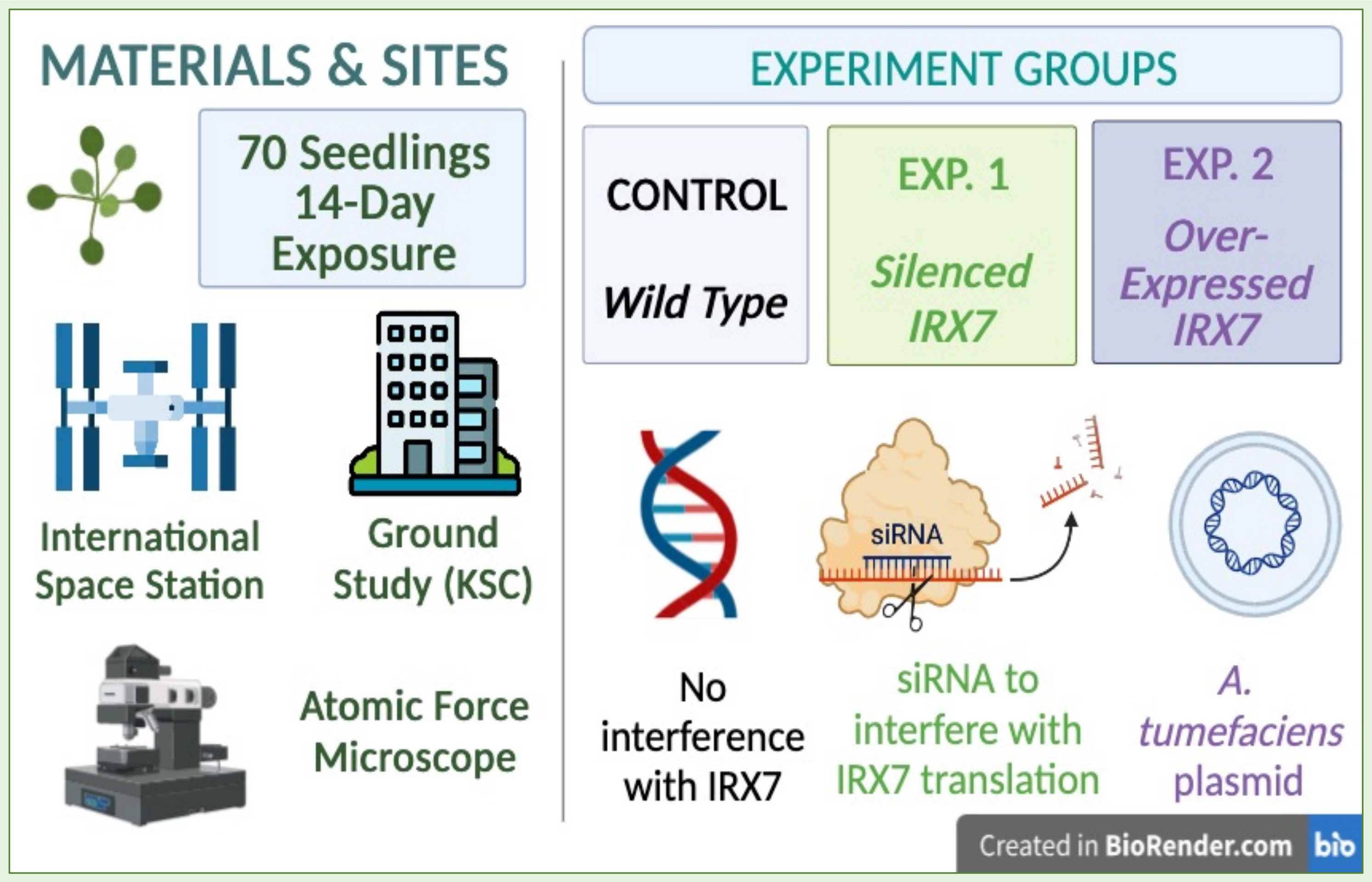


Figure 5: Silencing IRX7

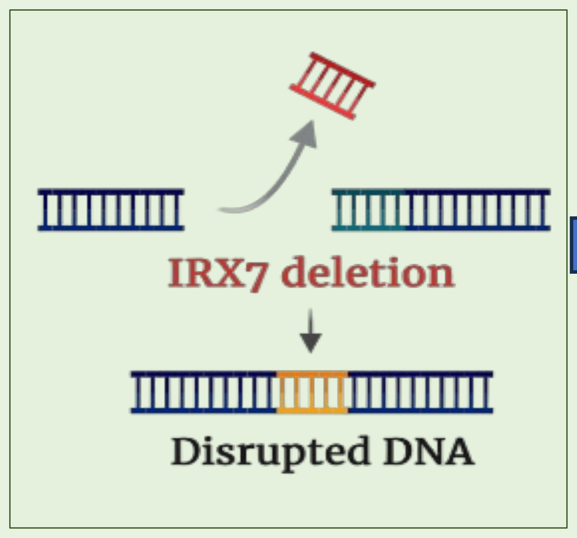


Figure 6: mutant

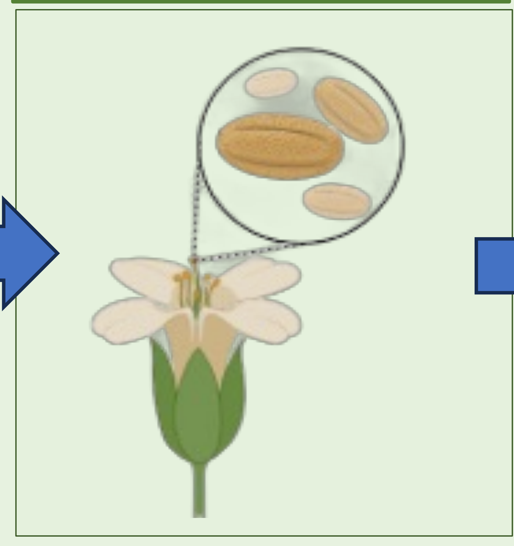
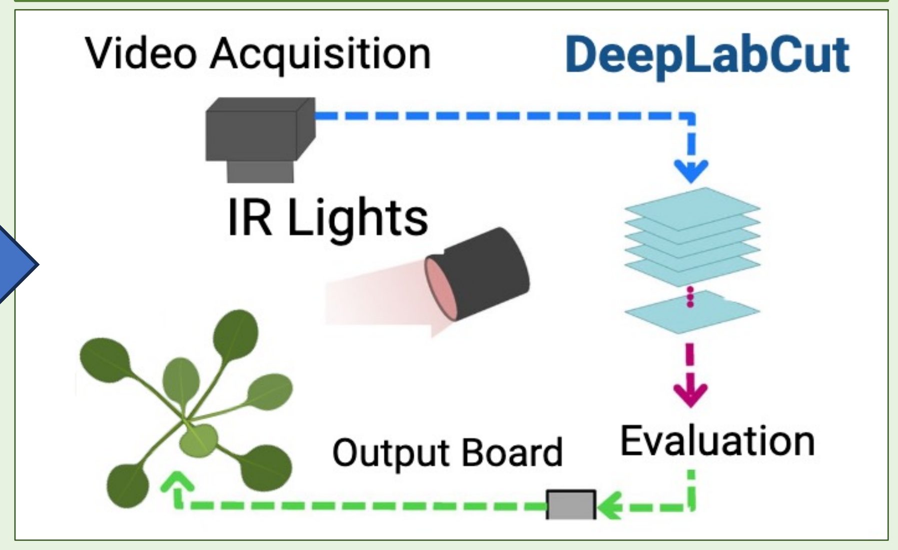


Figure 7: Machine Learning



- **Figure 4**: The relationship between UPR and CWI will be studied in terms of two genes: Gene bZIP28 (a key transcription factor in UPR) and Gene IRX7 (Irregular Xylem Gene7, oversees secondary cell wall biogenesis).
- **Figure 5, 6, 7**: *DeepLabCut* will quantify plant morphology in real-time (BRIC camera captures plant growth stages) and provide data to verify:
  - When silencing IRX7, UPR will be downregulated, leading to cell wall damage & weaker seedlings
  - When overexpressing IRX7, UPR will be upregulated, leading to stronger cell walls & seedlings.

Expected Results & Significance

- Without IRX7, the cell wall cannot repair itself. With over-expressing IRX7, the cell wall becomes more resilient to stressors.
- Altered plant architecture or reduced seed production may prevent the fruition of edible biomass and nutrient content and production necessary to sustain astronauts in extended spaceflight.
- As prepackaged foods may degrade in quality and nutrients during long-duration space missions, having resilient spaceflight-grown crops to supplement space diets with optimal nutrients and dietary antioxidants is essential to sustain life and work in space (Massa, et al., 2015).

Acknowledgements

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