Uncovering Unique Molecular Adaptations in the *Arabidopsis thaliana* Cvi-0 Ecotype

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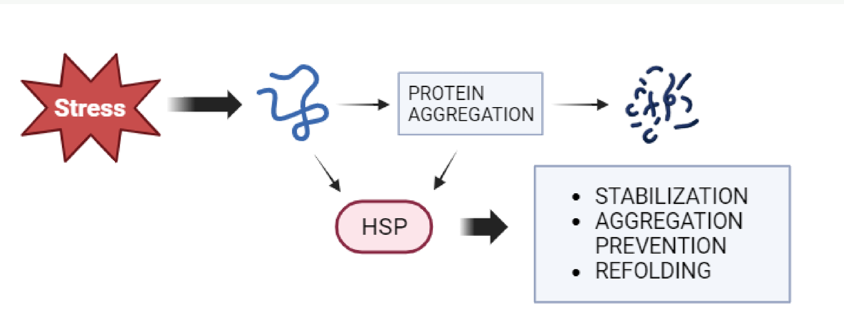
Introduction

Space colonization is an aspiration not too far in the future, and NASA's current goals for long-duration spaceflight missions may require astronauts to grow their own food. *Arabidopsis thaliana* is a model plant organism characterized by its short life cycle, small size, high reproducibility, and fully sequenced genome.Variations in gene expression, including upregulation of heat shock proteins (HSPs), have been observed in multiple ecotypes of *Arabidopsis* when subjected to the microgravity environment of space.

The specific mechanisms through which the *Arabidopsis* ecotypes adapt to spaceflight conditions, as well as genetic and molecular determinants that lead to ecotype-specific responses, remain largely unexplored. This research study aims to bridge these gaps by investigating four specific *Arabidopsis* ecotypes genomic response and unique adaptations of the *Arabidopsis* Cvi-0 ecotype in microgravity conditions, elucidating the roles of HSPs and RCA.

Background Research

Genes of interest in our investigation include HSP90-1, HSP70-3 and HSP70-5, MED37C, CLPB1, and RCA.



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HSPs are associated with cellular stress response and play roles in maintaining protein homeostasis under various environmental challenges. As these stressors attack the cell, heat shock factors activate HSPs through trimerization and subsequent gene expression. HSPs are mainly involved in regulation of gene expression, the refolding of proteins which have been denatured by stress, and stabilization of photosynthetic machinery.

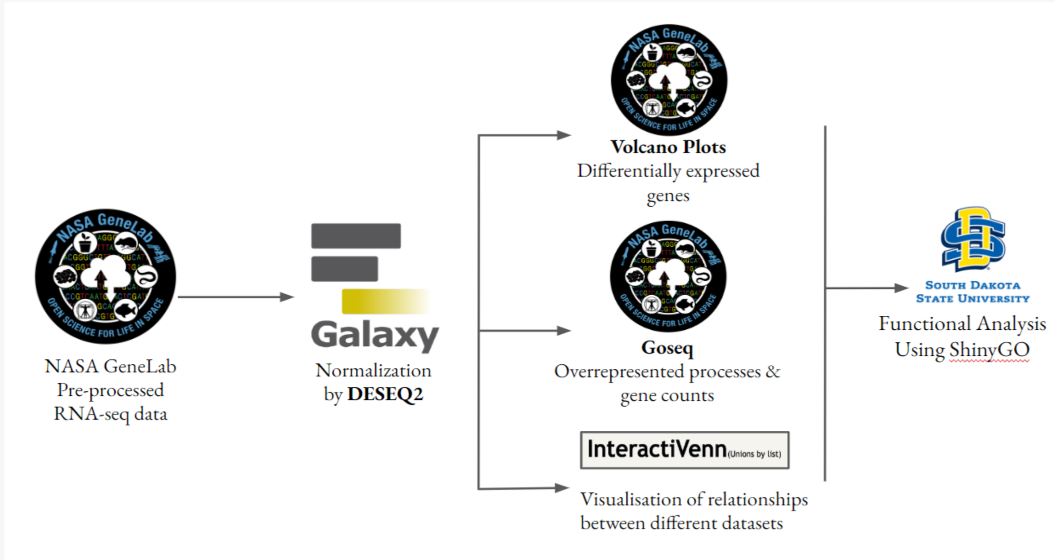
Rubisco activase (RCA) is an enzyme involved in modulating the activity of ribulose-1,5-bis-phospate carboxylase/oxygenase (Rubisco). Rubisco transforms carbon dioxide into organic compounds, thereby generating energy-rich molecules vital for plant growth and survival. Environmental stress can compromise the efficiency of photosynthesis due to the rapid deactivation of rubisco.

Methods

This study examines data from NASA's Open Science Data Repository consisting of 4 *Arabidopsis thaliana* ecotypes Col-0, Ler-0, Ws-2 and Cvi-0 (OSD-37,2015). The original experiment involved sending seeds of these four ecotypes to the International Space Station (ISS) via the BRIC-19 hardware, where they germinated and grew for 8 days in the absence of light. Ground control samples, grown on Earth, were used for comparison. RNA analysis performed per protocol.

Transcriptomic Data Analysis

Multifaceted RNAseq analysis pipeline performed using tools in the UseGalaxy.org open platform for our data analysis. Our preparation of differentially expressed genes involved file annotation and subsequent filtration of the counts table for statistical and biological significance.



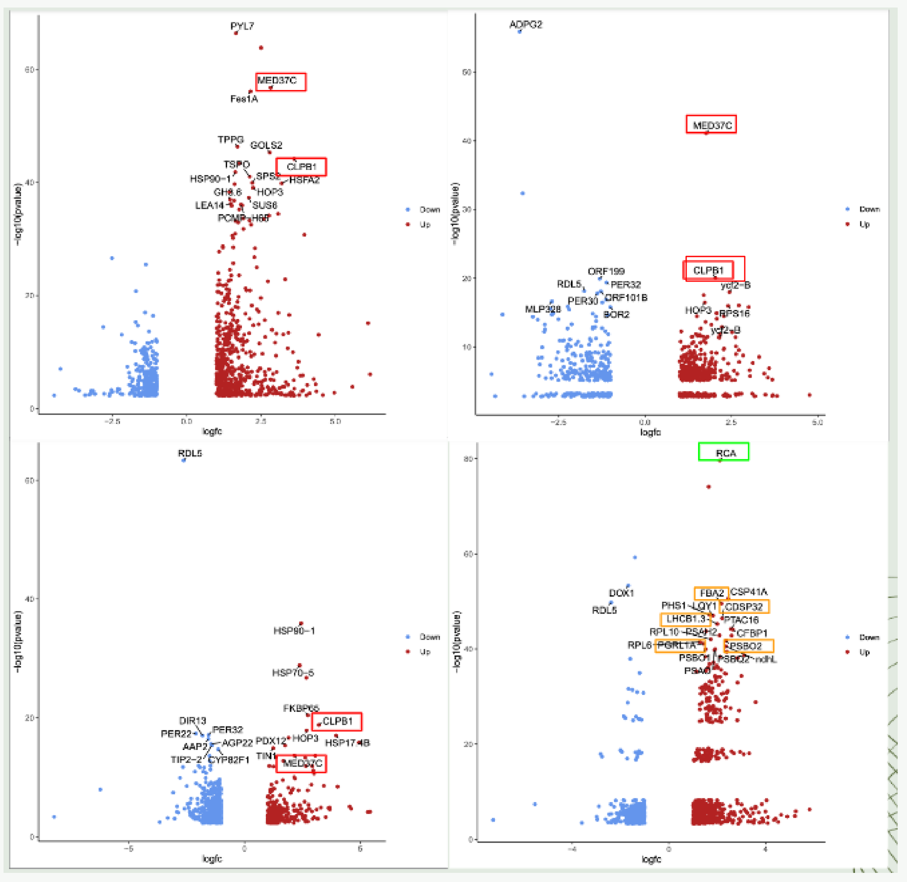


Figure 1- Volcano plots generated using the processed files within the Galaxy platform

Relations with HSPs

Roles in photosynthesis

Upregulation of RCA

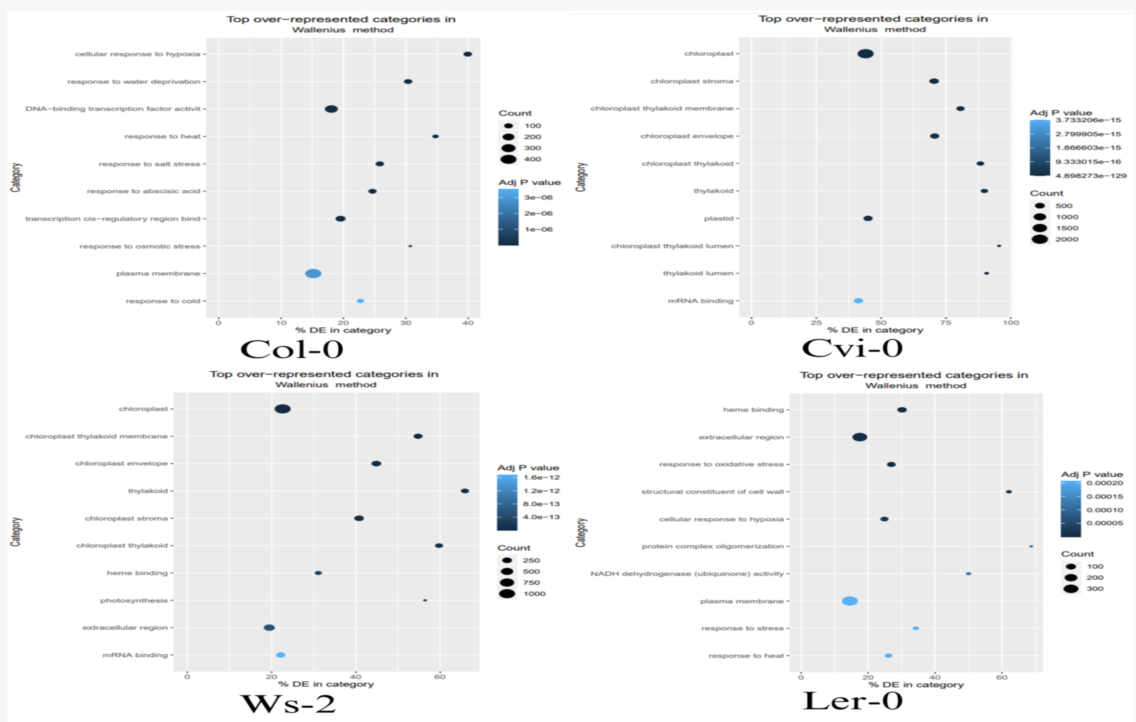


Figure 2- Over-represented GO terms plots

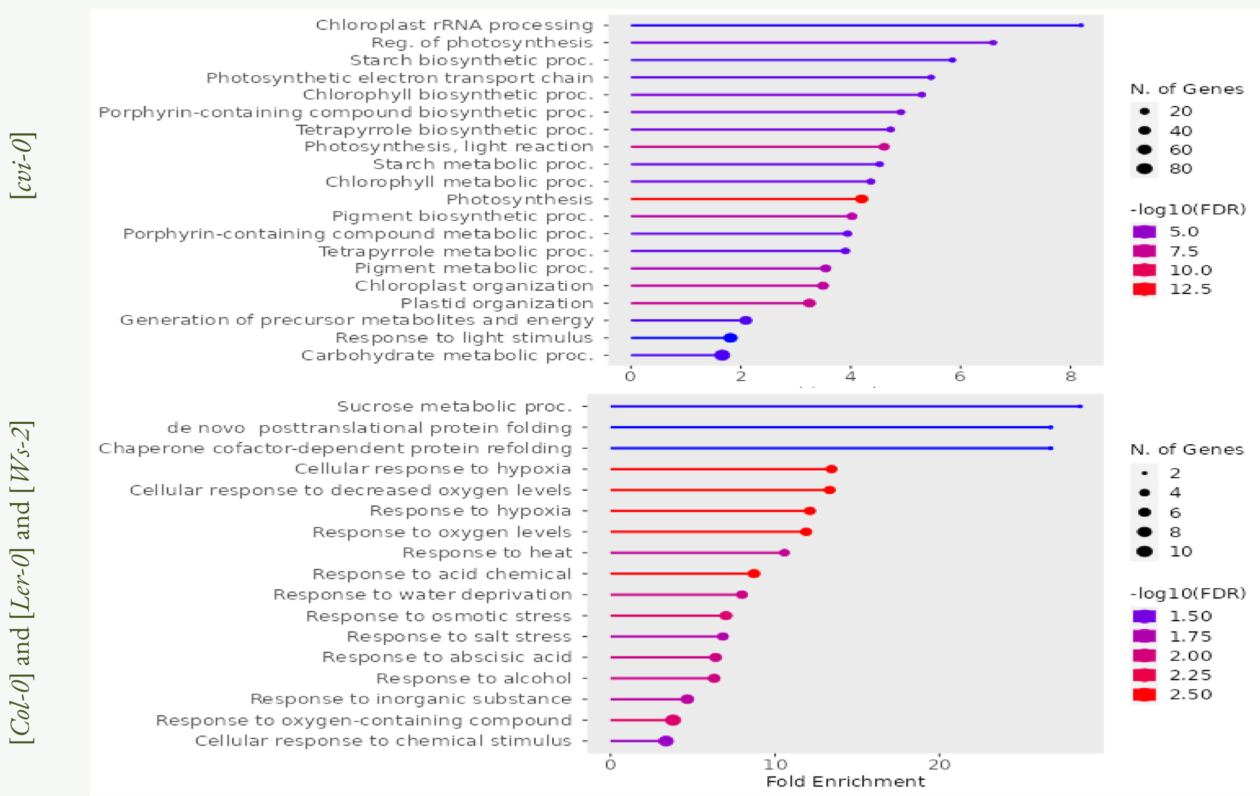


Figure 3- Functional analysis using ShinyGO demonstrated chloroplast response in upregulated in Cvi-0

Anticipated Results

Col-0, Ler-0 and Ws-2 ecotypes demonstrated upregulations of some or all of the following heat shock genes HSP90-1, HSP70-3, HSP70-5, MED37C and CLPB1 in spaceflight condition. Cvi-0 demonstrated an absence of heat shock protein (HSP) upregulation and an upregulation of rubisco activase (RCA) and chloroplast-related pathways.

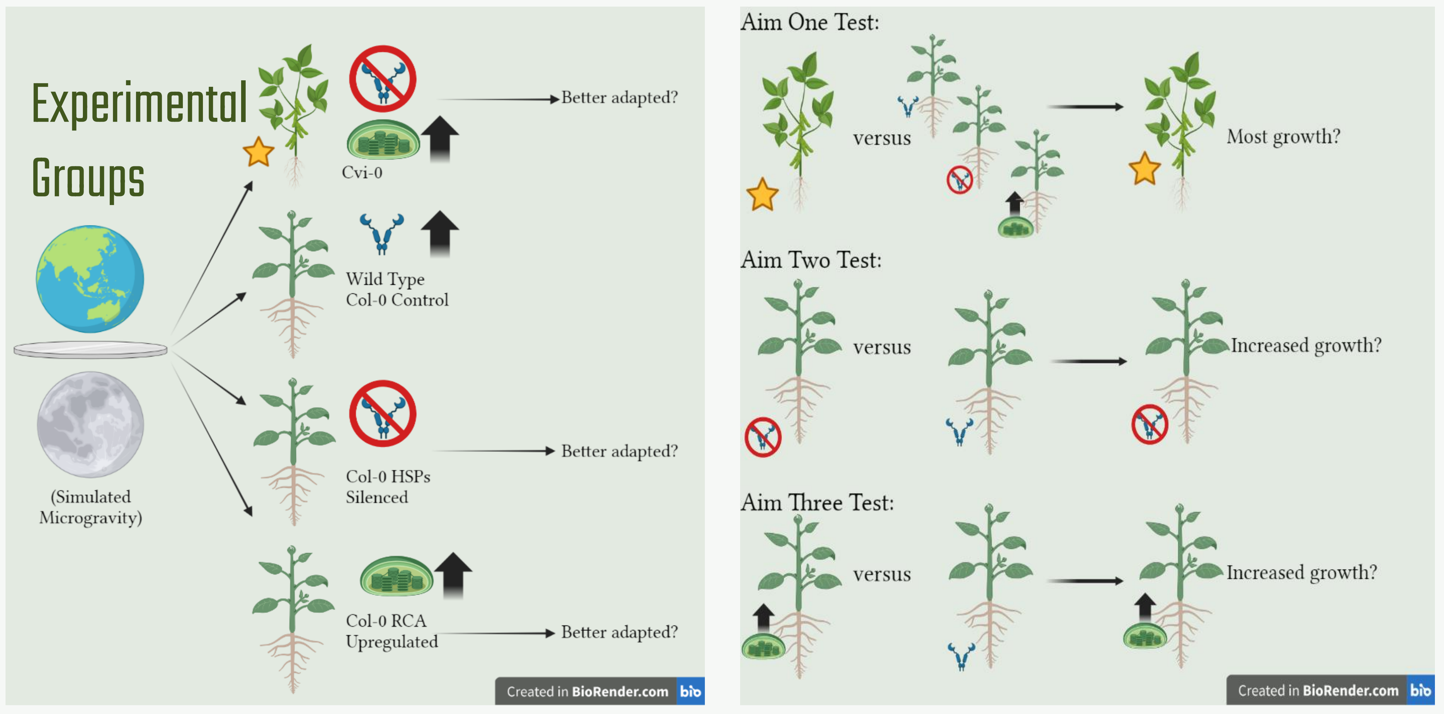
Consequently, we postulate that Cvi-0 may have already had adaptations suited for microgravity conditions. We thus hypothesize that the lack of upregulation of HSP genes, and the upregulation of photosynthetic pathways and related chloroplast functions involving RCA contribute to the Cvi-0 ecotype's molecular adaptation in microgravity conditions.

Future Direction: Proposed Methods

Aim One: To test that Cvi-0 is better adapted than Col-0 in simulated microgravity conditions using growth parameters as a measure of adaptability

Aim Two: To test Col-0 with silencing of genes MED37C, HSP90-1, HSP70-3, and CLPB1 to see if it has increased adaptability and if the silencing of HSP genes will elicit an alternative chloroplast stress response with RCA.

Aim Three: To test Col-0 with the upregulation of the RCA gene to see if it has increased adaptability and will elicit an alternative chloroplast stress response.



Significance

The envisioned experiment would play a pivotal role in narrowing the knowledge gap concerning the distinct adaptations exhibited by various *Arabidopsis* ecotypes, specifically focusing on the potential adaptations of the Cvi-0. It is imperative to account for the divergent responses of each ecotype, as one may display superior adaptability. This would allow us to target specific pathways, leading to more superior engineered crops. This proposed undertaking holds promise for fostering the development of more robust crops in space, bolstering the nutritional resources available to astronauts, as well as promoting sustainable agriculture practices on Earth.

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NASA Space Biology Program: https://science.nasa.gov/biological-physical/programs/space-biology

GL4HS Program: https://genelab.nasa.gov/genelab/highschools

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