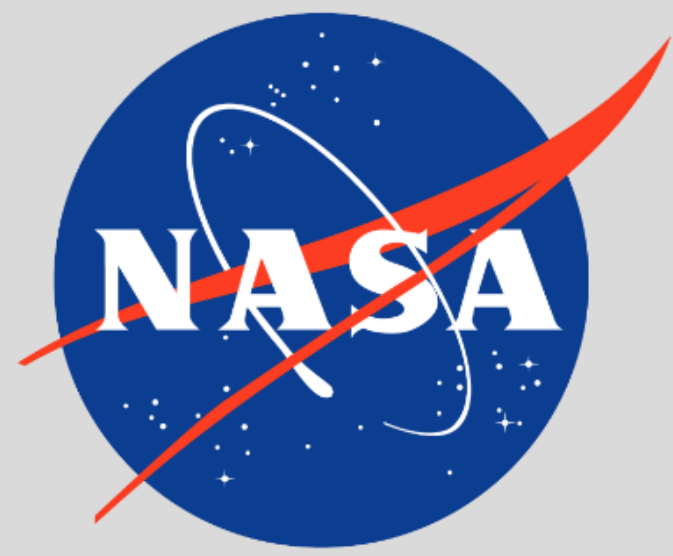




Oxygen Deficiency in Spaceflight & its Impact on Plants’ Adaptive Changes

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Introduction/Background

Spaceflight is an environmentally stressful condition for biological organisms because there is **a lower level of gravity, less oxygen availability, and greater cosmic radiation** as compared to on Earth (Manian, et al., 2021). It impacts plant growth as it induces stress. In *Arabidopsis thaliana*, **alcohol dehydrogenase (ADH)** is differently affected by redox modifications when under spaceflight stress due to transcriptome variation in **hypoxic conditions**.

Hypoxia is the term used to define **low levels of oxygen**, and plants respond to hypoxia with the expression of specific genes. The **genes that are induced by hypoxia** include ones that are specific for encoding the enzymes **needed for carbohydrate metabolism and fermentation**. Sugar availability in hypoxic conditions ensures that energy is produced (Loreti et al., 2017).

There are unique responses in the novel environment of spaceflight with heat shock factors and **heat shock protein genes response** from *Arabidopsis thaliana* cell cultures in spaceflight.. The root apex in *Arabidopsis thaliana* is impacted due to **root zone hypoxia**. Root zone hypoxia also occurs in *Brassica rapa* due to the lack of oxygen in the spaceflight environment.

Research Question/Hypothesis

This research study **compares the impact of hypoxia**, or oxygen deficiency, in two plant species, *Arabidopsis thaliana* and *Brassica rapa* from **previous spaceflight data** to understand its impact on **plant adaptive changes** specifically with gene expression.

Research question: Which genes in the two plant species are impacted by oxygen deficiency during spaceflight?

Hypothesis: If both plant species are exposed to hypoxic conditions in spaceflight environment, then the genes that will be impacted will be alcohol dehydrogenase and the heat shock proteins.

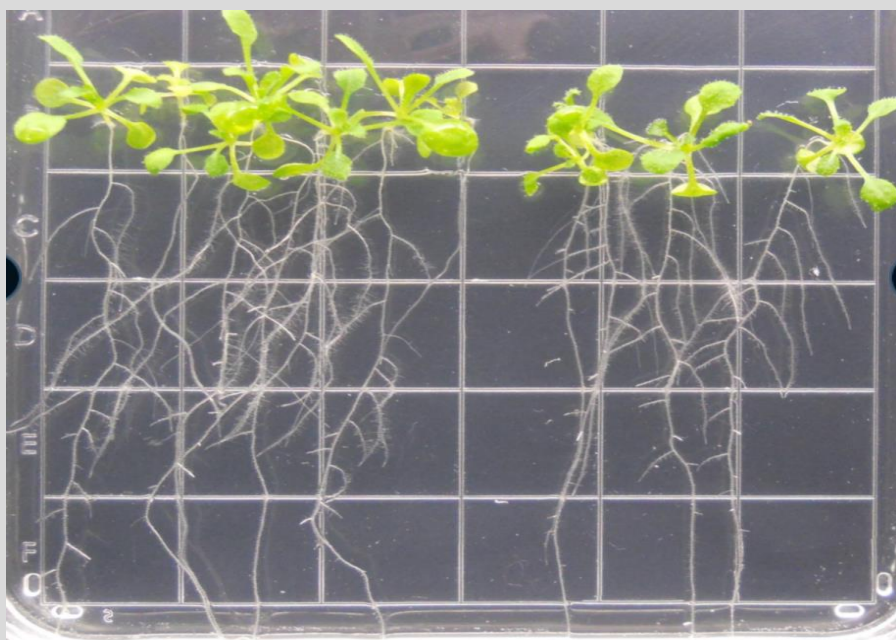


Image 1: An image of *Arabidopsis thaliana* (NASA Images)



Image 2: An image of *Brassica rapa* (NASA Images)

Plant Species Relation to Mammalian Species on Hypoxia

The study of low oxygen levels is also present in **mammalian species** as **respiratory long-term facilitation (LTF)** which is brought about by **hypoxia only** when it is present in an episodic pattern (Feldman, et al., 2003). Respiratory LTF is the long-lasting increase in ventilation lasting more than an hour which can be created by hypoxia or the electrical stimulation of the carotid sinus nerves (Feldman, et al., 2003). **Short-term chronic hypoxia** impacts gene expression especially for the ones encoding **heat shock factors, glycolytic enzymes**, cytoskeletal factors, extracellular factors, cell cycle regulators, angiogenic factors, and apoptotic factors (Ning et al., 2004). The information presents a correlation to plants because the plants grown in spaceflight conditions experienced increased levels of stress and reduced development.

Methods

First step: The first month of the project was dedicated to finding literature related to hypoxia, *Arabidopsis thaliana*, *Brassica rapa*.

Second Step: Journal articles related to hypoxia, *Arabidopsis thaliana*, *Brassica rapa* were read and annotated.

Third Step: A list of genes impacted by hypoxia was created from the readings.

Fourth Step: The NASA GeneLab Database was utilized to find transcriptomes data of two different spaceflight studies of *Arabidopsis thaliana*. Data systems GLDS-7 and GLDS-17 were compared.

Fifth Step: The transcriptomes were analyzed for GLDS-7 and GLDS-17 with the Test of Arabidopsis Space Transcriptome (TOAST) database. The genes that were considered significant had p-values ≤ 0.05 and log fold change values ≤ -1 or ≥ 1 . Sixteen genes fulfilled the criteria.

List of Genes Related to Hypoxia
alcohol dehydrogenase
alcohol dehydrogenase
alcohol dehydrogenase
alcohol dehydrogenase
alcohol dehydrogenase
alcohol dehydrogenase
alcohol dehydrogenase
elongation factor
elongation factor
ethylene-responsive factor
ethylene-responsive factor
ethylene-responsive factor

GUS1
GUS2
GUS3
HSP17.4
HSP17.4-CIII
HSP17.6A
HSP17.6B-CI
HSP17.6C-CI
HSP17.6II
HSP22.0
NAP
NAP
RAP2.12
RD20

- Table 1:** List of 16 genes (26 genes if repeats counted) related to hypoxia from GeneLab Data Systems GLDS-7 and GLDS-17
- Note: Some genes were represented more than once, so those are listed as well*

Sixth Step: The application RStudio was used to create graphs to analyze the results from the data systems.

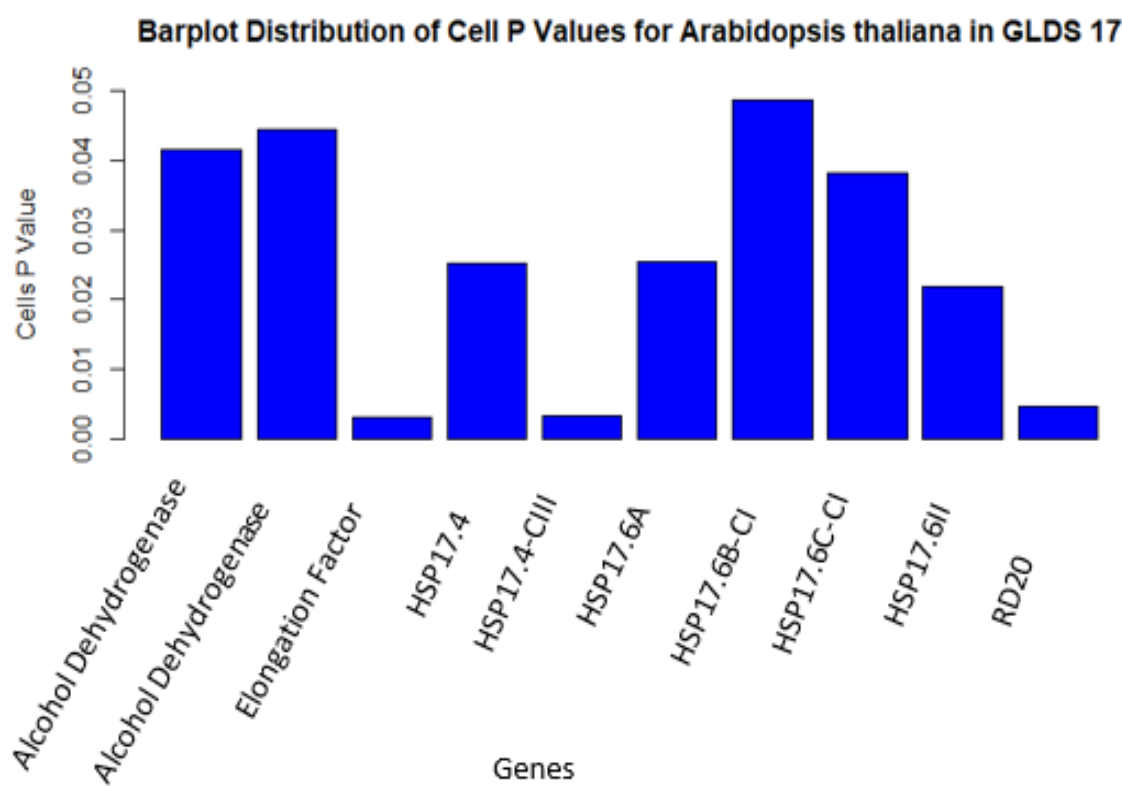
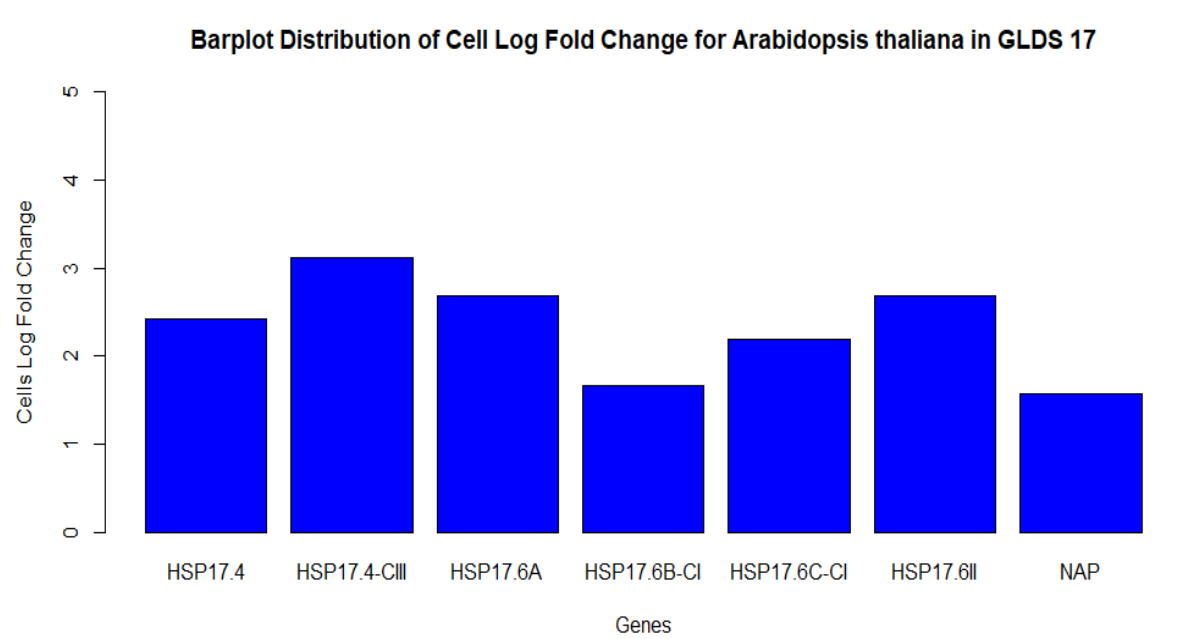
Seventh Step: These genes from *Arabidopsis thaliana* were compared with *Brassica rapa* through Arabidopsis Ensemble Orthology from the TOAST Database.

Eighth Step: After comparing the genes from *Arabidopsis thaliana* to *Brassica rapa* in hypoxic conditions, there was the comparison of similarities between mammals and plants and how they cope with hypoxia.

Results

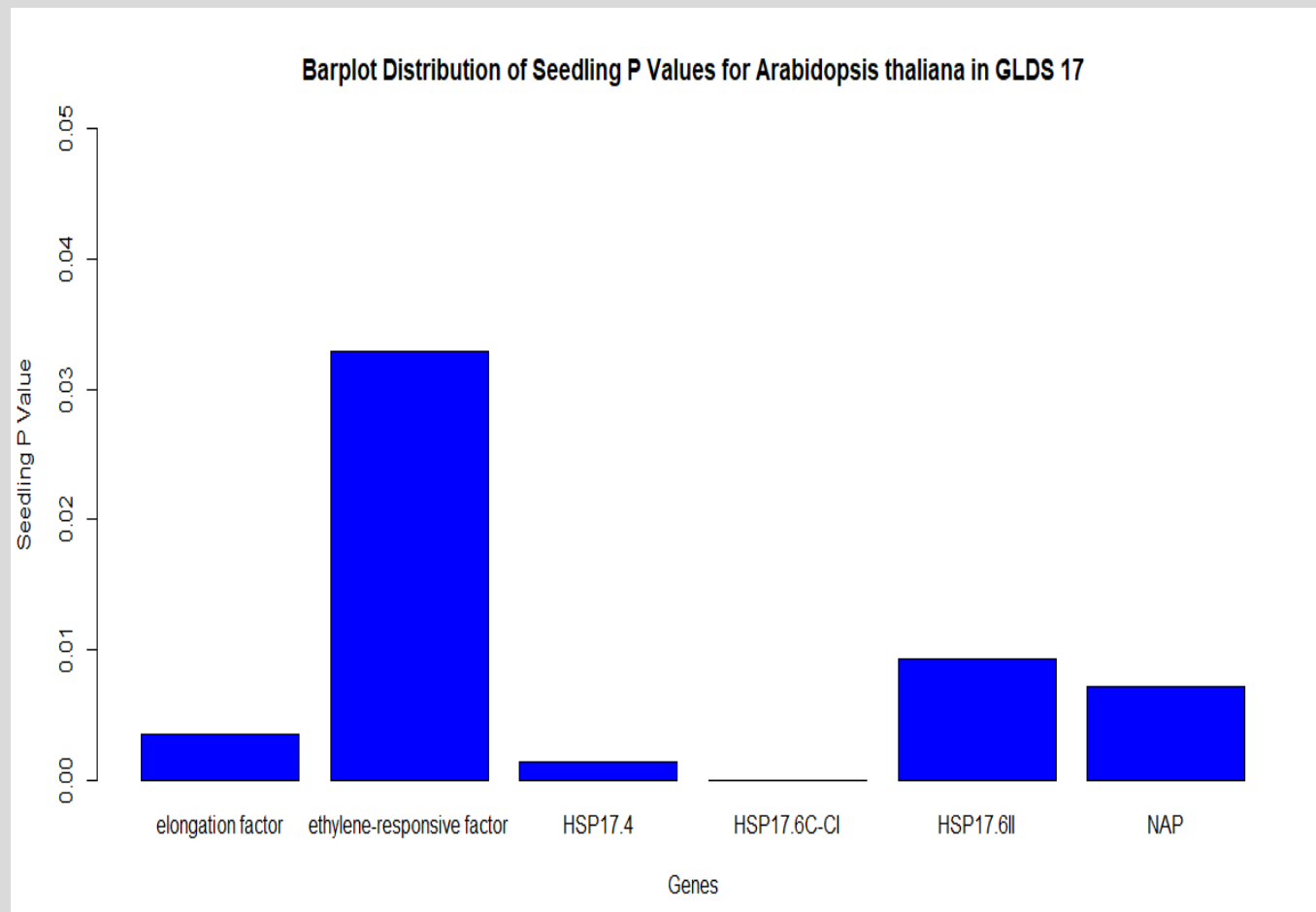
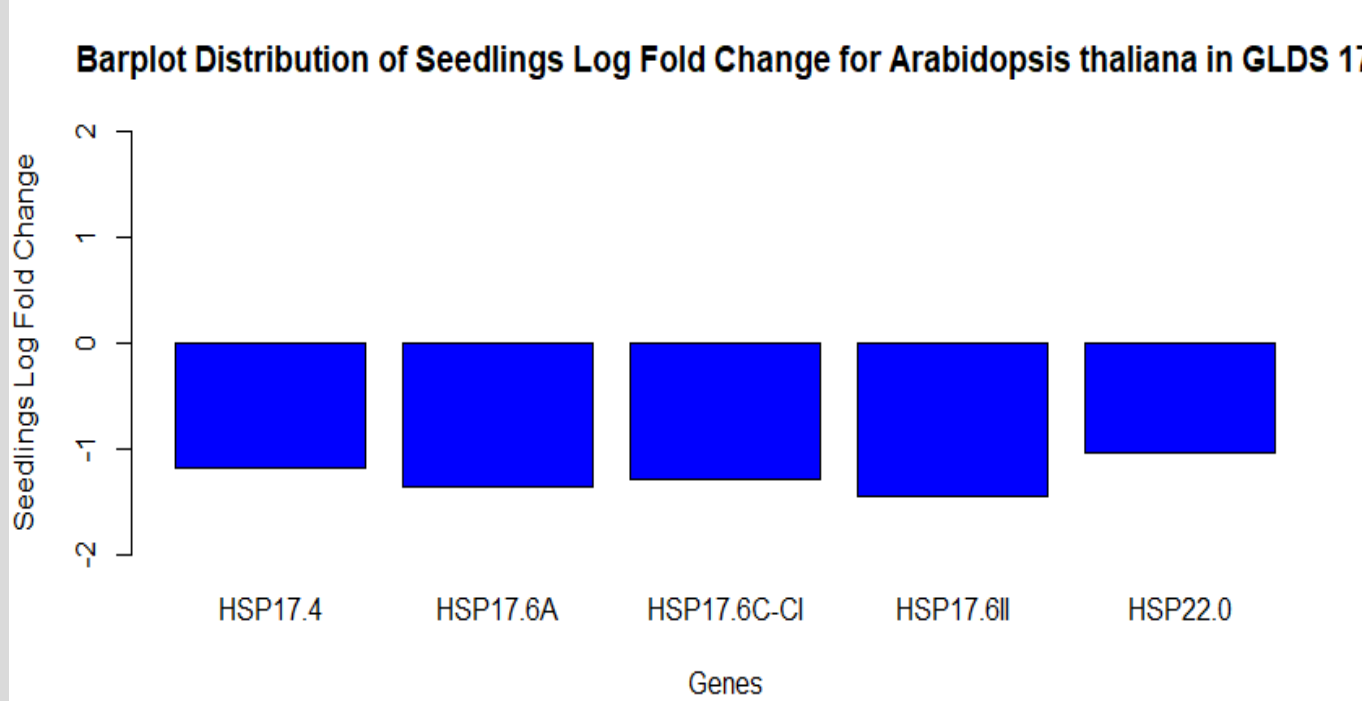
(GLDS-17)

Graph 1: Distribution of Genes involved with hypoxia in *Arabidopsis thaliana* with Cell P Value in GLDS-17



Graph 3: Distribution of Genes involved with hypoxia in *Arabidopsis thaliana* with Cell Log Fold Change in GLDS-17

Graph 2: Distribution of Genes involved with hypoxia in *Arabidopsis thaliana* with Seedling P Value in GLDS-17

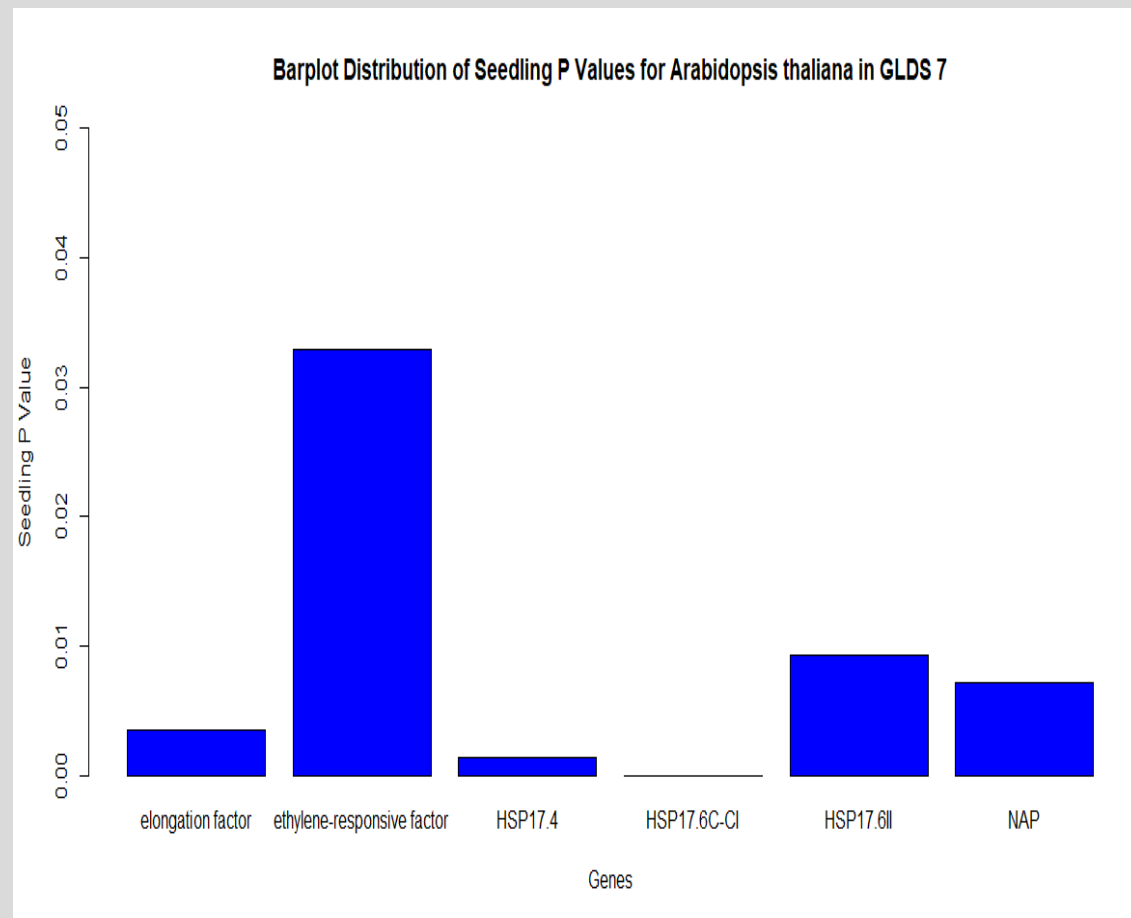
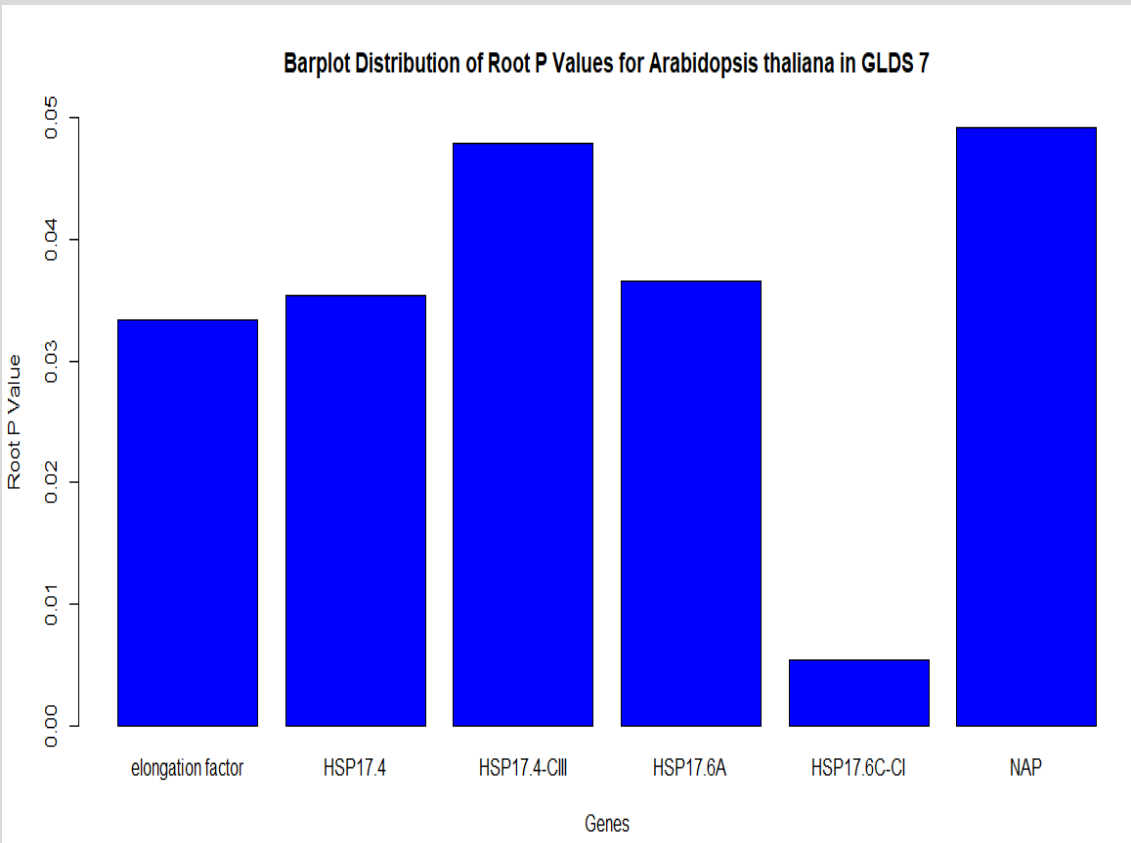


Graph 4: Distribution of Genes involved with hypoxia in *Arabidopsis thaliana* with Seedlings Log Fold Change in GLDS-17

Results

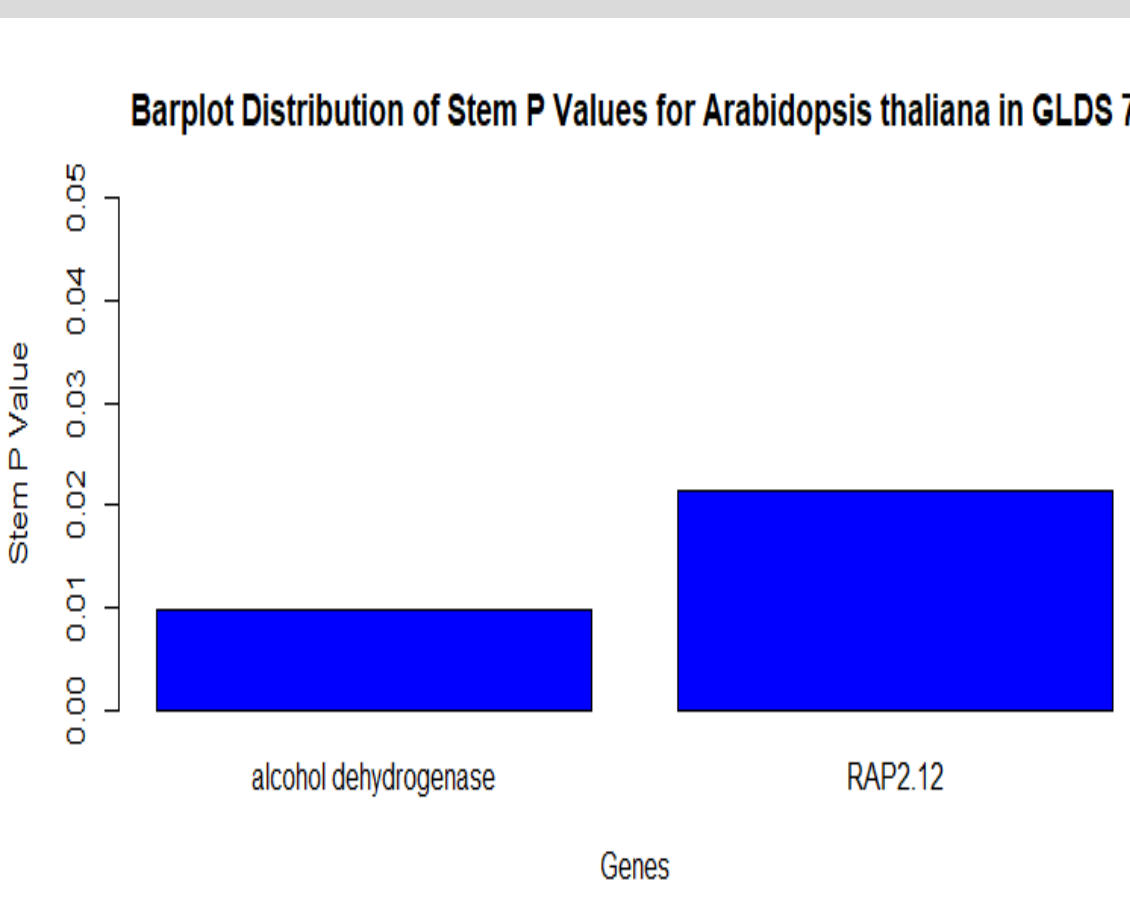
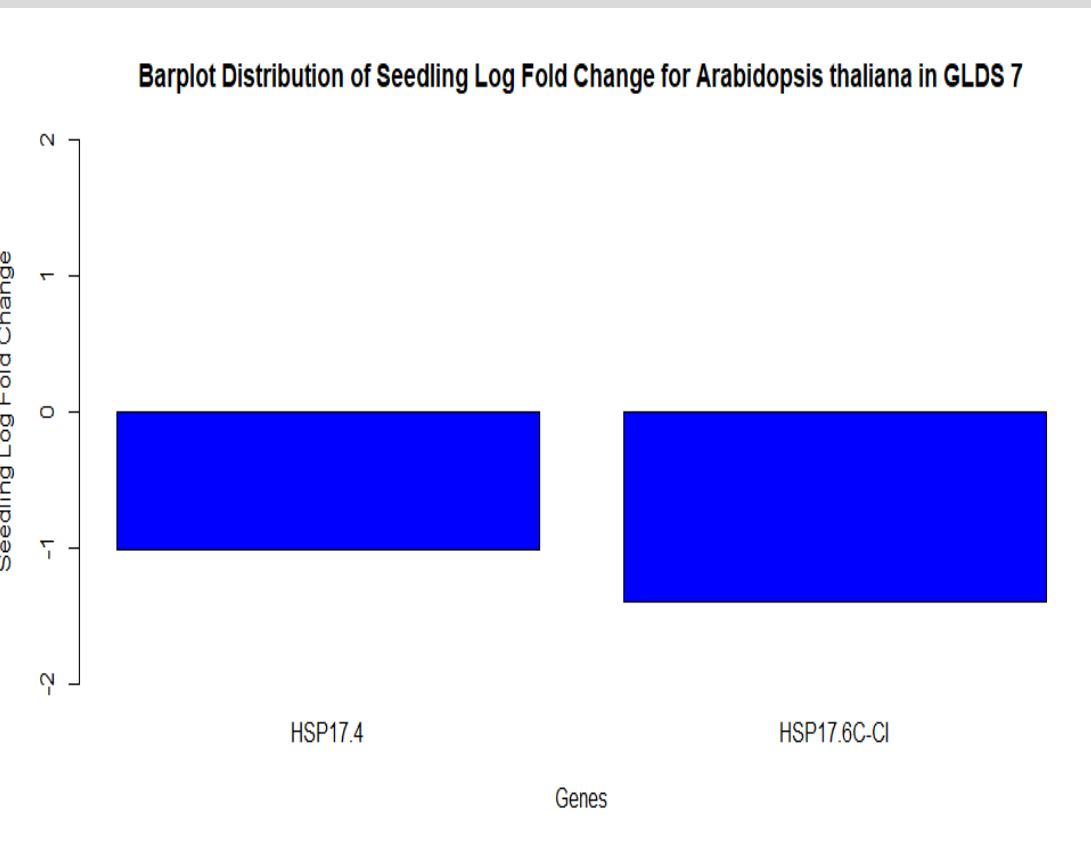
(GLDS-7)

Graph 5: Distribution of Genes involved with hypoxia in *Arabidopsis thaliana* with Seedling P Value in GLDS-7



Graph 7: Distribution of Genes involved with hypoxia in *Arabidopsis thaliana* with Root P Value in GLDS-7

Graph 6: Distribution of Genes involved with hypoxia in *Arabidopsis thaliana* with Stem P Value in GLDS-7



Graph 8: Distribution of Genes involved with hypoxia in *Arabidopsis thaliana* with Seedling Log Fold Change in GLDS-7

Conclusion & Future Directions

Overall, this study demonstrates that the genes related to hypoxia were **alcohol dehydrogenase, elongation factor, ethylene-responsive factor, GUS, heat-shock proteins, NAP, RAP2.12, and RD20**. The genes most impacted by spaceflight were heat-shock proteins. When the genes from *Arabidopsis thaliana* were compared with *Brassica rapa*, similarities were seen in **alcohol dehydrogenase, elongation factor, ethylene-responsive factor, heat-shock proteins, NAP, and RAP2.12**.

Arabidopsis gene name	Arabidopsis gene stable ID	Arabidopsis gene description
	AT5G42250	Alcohol dehydrogenase-like 7
	AT5G42760	Alcohol dehydrogenase-like 6
	AT5G13650	Elongation factor family protein
ERF096	AT5G43410	Ethylene-responsive transcription factor ERF096
HSP17.4A	AT3G23230	17.5 kDa class I heat shock protein
HSP17.4B	AT1G54050	17.4 kDa class III heat shock protein
HSP17.7	AT6G12030	17.7 kDa class II heat shock protein
HSP22.0	AT4G10250	22.0 kDa heat shock protein
NAC029	AT1G69490	NAP
RAP2.12	AT1G53910	Ethylene-responsive transcription factor RAP2-12

There are similarities with regards to how plants and mammals react to hypoxic conditions. This information indicates that there can be further studies that analyze the molecular mechanisms that are impacted in mammalian systems due to hypoxia. Over time, we look forward to conducting more studies looking at sustainable food and nutrition for astronauts. These studies will be beneficial for not only the advancement of crop plants, but also for the improved understanding on how to maintain astronauts' psychological and physical health (Oluwafemi, et al., 2021).

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