**What is the title of your project?**

Exploring the Interplay of Microgravity and Light on Plant Gene Expression

**Describe the space biology problem or challenge that you propose to address. What is the question you are trying to answer? What makes it significant, relevant, and interesting?**

The scientific problem proposed to address in this project revolves around understanding the combined effects of microgravity conditions and varying light parameters on altered gene expression related to chloroplast biogenesis and sugar metabolism in plants. The key question to answer is how do microgravity conditions, coupled with different wavelengths, intensities, and positions of light, influence the expression of genes critical for chloroplast development and sugar metabolism in plants?

This problem is significant because understanding how microgravity and light parameters impact gene expression in chloroplast biogenesis and sugar metabolism holds relevance for space exploration, contributing to fundamental plant science, agriculture and biotechnology.

POR (Protochlorophyllide oxidoreductase): Microgravity alters POR kinetics, impacting chlorophyll biosynthesis, Light quality and intensity affect POR activity in chlorophyll synthesis.

CHLH/GUN5 (Mg-chelatase subunit H): Microgravity influences CHLH/GUN5 expression and magnesium chelation, Light conditions modulate CHLH/GUN5 activity by affecting substrate availability.

SIG (Sigma factor): Microgravity-induced changes affect SIG activity in chloroplast gene regulation, Light variations influence SIG-mediated responses during chloroplast biogenesis.

PEPC (Phosphoenolpyruvate carboxylase): Microgravity impacts PEPC expression and carbon assimilation, Light availability regulates PEPC activity in photosynthesis.

Rubisco (Ribulose-1,5-bisphosphate carboxylase/oxygenase): Microgravity alters Rubisco function, affecting carbon fixation, Light intensity and quality directly influence Rubisco activity and assimilation rates.

**State your hypothesis and explain your reasoning.**

**Hypothesis:**

The combination of microgravity and varying light parameters will disrupt gene expression patterns in chloroplast biogenesis and sugar metabolism pathways in plants. We predict that microgravity-induced changes in growth orientation, coupled with alterations in light stimuli, will lead to imbalances in chloroplast function and sugar metabolism.

**Expected Results:**

• Altered Gene Expression: Microgravity and light variations will significantly alter gene expression profiles related to chloroplast biogenesis and sugar metabolism pathways.
• Disrupted Coordination: Interaction between microgravity and light conditions will disturb the coordination between chloroplast biogenesis and sugar metabolism pathways, potentially leading to metabolic imbalances.
• Impact on Plant Growth: These disruptions are likely to affect plant growth, development, and metabolic processes, potentially resulting in non-optimal phenotypes.
Overall, this study aims to provide insights into the effects of microgravity and light on plant gene expression, contributing to our understanding of plant biology in space and guiding strategies for space agriculture optimization.

**Outline your experimental plan:**

- **Which tools from the GiS toolkit will you use to test your hypothesis? (50 words)**

Gene Expression Analysis: Collect plant tissue samples at specific times to capture dynamic gene expression changes; Extract RNA using the Genes in Space Toolkit's protocol; Perform qRT-PCR to quantify key gene expression levels; Compare gene profiles between control and experimental groups under varied light conditions.

**What samples will you test? (100 words)**

**Experimental Setup:**

• Grow Arabidopsis thaliana, commonly known as thale cress or mouse-ear cress and also different types of lettuces in International Space Station (ISS), to expose the experimental samples to microgravity conditions.
• Install controlled lighting systems to manipulate light parameters (wavelength, intensity, position) for each experimental group.
• Implement replicates for each experimental condition to ensure statistical robustness.

**What controls will you include? (100 words)**

**Controls:**

**Positive Control:** Include samples grown under normal gravity conditions as a positive control to establish baseline gene expression levels.

**Negative Control:** Implement samples grown under microgravity conditions without light manipulation to account for non-specific effects of microgravity.

**Technical Controls:** Include technical replicates and internal controls (e.g., housekeeping genes) to ensure experimental consistency and data reliability.

**Explain why you selected the tools you incorporated into your experimental plan.**

• **Genes in Space Toolkit for RNA Extraction:**

**Rationale:** RNA extraction is essential for analyzing gene expression patterns, and the Genes in Space Toolkit offers robust protocols tailored for space-based experiments.

**Fit for Research Question:** Provides a reliable method for extracting high-quality RNA from plant samples under microgravity conditions, ensuring accurate gene expression analysis.

• **qRT-PCR (Quantitative Reverse Transcription Polymerase Chain Reaction):**

**Rationale:** qRT-PCR is a highly sensitive technique for quantifying gene expression levels, allowing precise measurement of mRNA transcripts.

**Fit for Research Question:** Facilitates the assessment of altered gene expression in chloroplast biogenesis and sugar metabolism pathways under different experimental conditions.

- **Statistical Analysis:**

**Rationale:** Statistical analysis is crucial for interpreting experimental results and identifying significant differences in gene expression patterns.

**Fit for Research Question:** Allows for rigorous analysis of qRT-PCR data to determine the effects of microgravity and light parameters on gene expression, providing statistical validation of experimental outcomes.

**Citations (optional)**

1.Schmitt FJ, Campbell ZY, Bui MV, Hüls A, Tomo T, Chen M, Maksimov EG, Allakhverdiev SI, Friedrich T. Photosynthesis supported by a chlorophyll f-dependent, entropy-driven uphill energy transfer in Halomicronema hongdechloris cells adapted to far-red light. Photosynth Res. 2019 Mar;139(1-3):185-201. doi: 10.1007/s11120-018-0556-2. Epub 2018 Jul 23. PMID: 30039357.

2.Chen M, Hernandez-Prieto MA, Loughlin PC, Li Y, Willows RD. Genome and proteome of the chlorophyll f-producing cyanobacterium Halomicronema hongdechloris: adaptative proteomic shifts under different light conditions. BMC Genomics. 2019 Mar 12;20(1):207. doi: 10.1186/s12864-019-5587-3. PMID: 30866821; PMCID: PMC6416890.

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4.Zhao Q, Li J, Liu M. Effects of simulated microgravity on characteristics of photosynthesis in plant seedling. Space Med Med Eng (Beijing). 2002 Apr;15(2):79-83. PMID: 12066822.

5. Sugano M, Ino Y, Nakamura T. Growth and photosynthesis of Japanese flowering cherry under simulated microgravity conditions. Biol Sci Space. 2002 Dec;16(4):242-4. doi: 10.2187/bss.16.242. PMID: 12721527.