

Fiscal Year:	FY 2024	Task Last Updated:	FY 09/08/2023
PI Name:	Paul, Anna-Lisa Ph.D.		
Project Title:	Hypobaric Plant Biology in Space Exploration - Molecular Responses of Arabidopsis to Combined Effects of Low Atmospheric Pressures and Microgravity of Spaceflight Vehicles		
Division Name:	Space Biology		
Program/Discipline:			
Program/Discipline--Element/Subdiscipline:			
Joint Agency Name:		TechPort:	No
Human Research Program Elements:	None		
Human Research Program Risks:	None		
Space Biology Element:	(1) Plant Biology		
Space Biology Cross-Element Discipline:	None		
Space Biology Special Category:	None		
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Comments:			
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Flight Program:	ISS		
Flight Assignment:			
Key Personnel Changes/Previous PI:	Addition of a research scientist, Dr. Mingqi Zhou, who was added to to his transcriptomic and hypobaric studies in the past.		
COI Name (Institution):	Ferl, Robert Ph.D. (University of Florida, Gainesville)		
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Task Description:	<p>Atmospheric pressure and composition are among the engineering variables considered in the design and construction of spaceflight vehicles and extraterrestrial habitats. Simply put, the costs of maintaining a pressure vessel at one atmosphere have been traded away throughout the history of spaceflight vehicle design and are traded away in future designs. And while the effects of hypobaria on plant biology are now well understood, there exist no studies on the combined effects of hypobaria and microgravity. In other words, the dominant physical manifestation of spaceflight, microgravity, has been left out of our understanding of plant hypobaria. We therefore propose to examine plant responses and physiological adaptations to the combined effects of low pressure while in the microgravity of the International Space Station (ISS).</p> <p>The objective of this proposal is to develop a refined understanding of the metabolic processes involved in plant responses and physiological adaptations to low pressure environments within space exploration vehicles and habitats. The long-term goal of this line of research is a fundamental understanding of low pressure plant biology within exploration vehicles and structures, with a practical goal of contributing to the design of plants that thrive in challenging exploration environments. The essential drivers of this project are that hypobaric environments will likely be a feature of future exploration vehicles and habitats, together with the knowledge that plants mount complex and costly metabolic responses to hypobaria. Furthermore, plants mount complex and sometimes unexpected responses to spaceflight and altered gravity environments. We hypothesize that the combination of hypobaria and microgravity will have a synergistic effect on the physiological adaptation to this complex environment, and that the patterns of gene expression will reveal strategies to both understand and help ameliorate the combined effect. These responses will also inform fundamental understanding of how plants adapt to changing terrestrial habitats facing complex and novel stressful environments.</p> <p>There is a facility on the ISS that is well suited for these experiments: the Combustion Integrated Rack (CIR). The CIR has a pressure vessel that can be programmed telemetrically to the gas composition and pressures relevant to our study. There is also a CIR ground unit that can be similarly programmed for parallel ground controls, thereby enabling the precise dissection of the effects of the orbital environment on plant biology within the pressure vessel.</p> <p>The proposed research will provide fundamental insights into the biological impact of novel atmospheric environments, a focus area that is itself specifically identified in the Decadal Study. (Ed. Note. The National Academies of Sciences, Engineering, and Medicine Decadal Survey on Biological and Physical Sciences Research in Space 2023-2032). More importantly, this Hypobaric Plant Biology in Space Exploration study seeks to fundamentally examine combined spaceflight effects in order to develop an understanding of emergent response properties that are not predicable from an examination of the individual responses.</p>
Rationale for HRP Directed Research:	
Research Impact/Earth Benefits:	<p>This research contributes to a greater understanding of the role of transcriptomic changes to the plant in response to both hypobaric and microgravity environments. Further, it will be the first experiment to test whether the interplay between hypobaric stress to plants and microgravity growth of plants has synergistic effects.</p>
Task Progress:	<p>Seed line development The T-DNA insertion mutant line has had its knockout status for the PDC1 gene verified through expression monitoring, along with seed bulking. Expression monitoring was completed through a comparison of wildtype and mutant lines following a 4°C induction and quantitative reverse transcription polymerase chain reaction (qRT-PCR) analysis. This analysis displayed a distinct reduction in expression of the PDC1 gene, consistent with a knockout status. The green fluorescent protein (GFP) reporter lines are in varying stages of development. Two lines are currently undergoing expression verification tests to ensure visible production of the GFP protein. These lines have been confirmed using qRT-PCR analysis, and microscopy analysis to visualize the GFP protein and monitor its abundance. One line has been transformed and is at the transition from the T1 to T2 generations. This line was generated in-house through restriction enzyme mediated vector generation, which was produced using the promoter sequence cloned from the Columbia-0 background driving the expression of GFP. Screening of expression is currently being conducted on multiple lines to ensure presence and correct expression of the GFP protein. The inductions are carried out through crimping of leaves followed by microscopy. pPDC1:GFP is currently in vector development.</p> <p>Testing Short-duration hypobaric treatments have been conducted. These treatments are conducted using clear hypobaric chambers retrofitted with a MSR Electronics data logger, to track barometric pressure, and a vacuum pump to draw out the gases from the chamber. Treatments typically last for 12-24 hours and are subjected to varying reduced pressures from 10-80kPA. These are general atmosphere reductions, not controlled gases. The purpose of these treatments has been to examine expression of the reporter lines in a hypobaric environment. Further analysis of the pdc1 knockout line using the hypobaric chambers, followed by expression verification through qRT-PCR, is also currently underway.</p> <p>Hardware Progress The hypobaric experiment, which has been designated APEX-11 (Advanced Plant Experiments), is scheduled to use the CIR (Combustion Integrated Rack) facility and the VEGGIE growth chamber. The plants will grow for 10 days in the NASA Vegetable Production System (Veggie growth chamber) using a still-under-development plant growth rack. The Veggie rack will be designed for 40 plates, which will allow for a shortened experimental procedure process. There is also currently a separate rack in development for hypobaric treatments inside the CIR. In April 2023, Dr. Paul traveled to NASA Glenn Research Center outside of Cleveland, OH to work on a plan to integrate science into the CIR. Dr. Paul traveled with several plates to serve as mockup plates that will grow the plants for APEX-11. These plates were used to test the prototype rack. Plates were mounted on the rack and loaded into the CIR for design tests.</p> <p>Future Plan Current plans call for Science Verification Test (SVT) to being in Q4 2023, with Experiment Verification Test (EVT) and Flight operations in 2024. SVT stage of operation is ready to move forward on the biology side; however, hardware operations are still in planning stages.</p>
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