

Fiscal Year:	FY 2022	Task Last Updated:	FY 05/03/2022
PI Name:	Lewis, Norman G Ph.D.		
Project Title:	An Integrated Omics Guided Approach to Lignification and Gravitational Responses: The Final Frontier		
Division Name:	Space Biology		
Program/Discipline:			
Program/Discipline--Element/Subdiscipline:	SPACE BIOLOGY--Developmental biology		
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Human Research Program Risks:	None		
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Comments:			
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No. of Bachelor's Candidates:	1	Monitoring Center:	NASA KSC
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Key Personnel Changes/Previous PI:			
COI Name (Institution):	Davin, Laurence Ph.D. (Washington State University) Hanson, David Ph.D. (University of New Mexico) Lipton, Mary Ph.D. (Battelle Memorial Institute) Sayre, Richard Ph.D. (New Mexico Consortium) Starkenburger, Shawn Ph.D. (Los Alamos National Security)		
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Performance Goal No.:	
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Task Description:	<p>We propose a multi-omics study using the model plant <i>Arabidopsis</i> under both 1g and in microgravity conditions (spaceflight). Our approach spans comprehensive phenomics, metabolomics, transcriptomics, and proteomics strategies, and is incisively and uniquely melded via deployment of an integrated computational biology (ICB) approach we are pioneering. Our plant lines include wild type, various mutants we have generated with different lignin amounts through manipulation of the multigene family encoding arogenate dehydratases, and other lines enhanced in carbon assimilation capacity, and combinations thereof. We consider this places us in an unprecedented position to investigate how plants function in altered gravity environments.</p> <p>We are very well positioned for incisive spaceflight and definition stage (1g) studies to investigate gene/metabolic network relationships and adaptations resulting from varying lignin and carbon assimilation levels, e.g., on photosynthesis; C allocation; water use efficiency (WUE); vascular plant growth/development; vasculature performance; auxin transport; and gravitational adaptations. Our overarching hypothesis is that a comprehensive interrogation (an integrative omics study) of our <i>Arabidopsis</i> lines with varying lignin levels and/or modulated carbon concentrating mechanisms (CCMs) or combination thereof will identify gene/metabolic networks, mechanisms and/or pathways that are differentially modulated at 1g and on exposure to microgravity, i.e., various omics (phenomics, transcriptomics, genomics, proteomics, metabolomics, and ICB) will allow us to study these in a truly unprecedented way.</p> <p>Overall objectives:</p> <ol style="list-style-type: none"> 1. Establish multi “omics” effects of modulating lignin and CCM levels i) at 1g and ii) in spaceflight. 2. Compare/contrast data, using an ICB approach, to better define and understand gravity sensing and responses, and if threshold/induction parameters are modified or changed, when lignin and CCM levels are varied. <p>More specifically, we address distinct hypotheses for our various teams, and integrate, dissect, and incisively analyze them holistically in a manner hitherto not possible. These 5 hypotheses include that: modulating lignin and CCM levels differentially affect carbon assimilation/re-allocation, photosynthesis, and WUE (Team 1); modulating lignin and CCM levels differentially affect secondary and primary metabolite levels (metabolomics) (Team 2); system-wide modification in the transcriptome occurs through a common transcriptional regulatory mechanism, and transcriptome/proteome “discrepancies” result from over-simplification of transcript analyses (Team 3); differential alterations in lignin and CCM levels can often be attributed to overall distinct changes in protein expression and phosphorylation patterns in a defined set of proteins (Team 4); an integrated omics analysis will provide urgently needed new insights into global effects on plant biological processes at both 1g and in microgravity (Teams 1-4). Each hypothesis draws upon the most advanced technologies available for study. We consider that our ICB approach will transform omics analysis through our advanced instrumentation and analytical tools. We will utilize (or design) computational tools/mathematical algorithms for integration and correlation of high resolution phenotype measurements (phenomics) with 'low' resolution global subcellular system measurements (transcriptomics, etc.) through 'nth' dimensional analysis.</p> <p>Our study aligns with Research Emphasis 1 and 3, and decadal survey elements in Cell, Microbial, and Molecular Biology (CMM-3, CMM-5), Organismal and Comparative Biology (OCB 2-5), Developmental Biology (DEV-4), and Plant and Microbial Biology, chapter 4 (P2). Our data generation will also be seamlessly integrated with various web-based platforms to handle, disseminate, and inter-actively utilize through iPlant and OpenMSI, and thus are made available to NASA as well as being a community resource.</p>
Rationale for HRP Directed Research:	
Research Impact/Earth Benefits:	<p>This research will provide NASA and science in general with the first “big ‘omics’ data” analysis, integration, and assessment – at the gene, protein, and metabolic outcome levels – as to how microgravity alters the basic responses of plants when the influence of gravity is removed/minimized. This will serve as the foundation “omics” analyses in subsequent spaceflight and colonization experiments in space, as well as shedding new insights into the manifold effects of gravity during plant growth and development.</p> <p>Presentation to Science Mission Directorate: Lewis, NG. "Multi-omics analyses of lignin reduced <i>Arabidopsis</i> lines on ISS." NASA's Associate Administrator of the Science Mission Directorate (SMD), Washington DC, December 2, 2021. This presentation described several of the exciting new insights gained from the ISS studies.</p>
	<p>Our ongoing investigation involved two <i>Arabidopsis</i> grow-outs on the International Space Station (ISS), with plants grown and monitored in the Advanced Plant Habitat (APH), with frozen specimens returned to Earth for multi-omics analyses. Corresponding APH Ground controls were carried out at NASA Kennedy Space Center (KSC) by KSC-affiliated collaborators. Multi-omics analyses were done in collaboration with Washington State University (WSU), University of New Mexico (UNM), Pacific Northwest National Laboratory (PNNL) and Los Alamos National Laboratory (LANL) consortium members.</p> <p>The overall study had 6 <i>Arabidopsis</i> lines, one wild type (WT), a transgenic line expressing a protein involved in the carbon capture mechanism (CCM), as well as four lines of lignin-reduced arogenate dehydratase mutants (adt), with these encompassing a single mutant (adt5), a double mutant (adt4/5), a quadruple mutant (adt3/4/5/6), and the adt3/4/5/6 mutant expressing CCM.</p> <p>Photosynthesis measurements on the ISS (and also KSC) deployed portable handheld pulse-amplitude modulated (PAM) devices, these being the first PAM photosynthesis measurements in low Earth orbit (LEO). [PAM measurements estimate the efficiency with which photosystem II (PSII) utilizes light energy for individual plants.]</p> <p>Relative growth and health of the various <i>Arabidopsis</i> lines in the APH (on either the ISS or at KSC) were monitored through assessment of daily changes in rosette size area and plant color, this being recorded/measured telemetrically. These showed a consistent pattern of slower rosette growth on the ISS relative to KSC Ground Controls. Flowering stem growth estimates and their abilities to have an upright (vertical/near vertical) orientation were also assessed. As flowering progressed, most main flowering stems and subsidiary stems on the ISS in both APH Grow-outs deviated unusually from vertical growth.</p>

Task Progress:	<p>Lignin levels were quite similar for KSC Ground Control WT and CCM lines, whereas adt mutants showed increasingly lower lignin contents depending on the mutant. Although a similar trend was noted in ISS plant lines, their levels were further reduced due to plant growth/development/lignification delays. However, reduced lignin levels did not result in vertical stem orientation, suggesting that such levels are not compensated for in microgravity on the ISS. Anatomical analysis of available ISS stem cross-sections indicated some vasculature abnormalities, as compared to the KSC Ground Controls.</p> <p>Proteomics and Transcriptomics data were obtained for both Grow-outs, together with metabolomics (including lipidomics) analyses). Meaningful integration of multi-omics data is a well-known challenge across all biology disciplines. Accordingly, our inter-disciplinary team effort is largely now focusing on integration and analysis of these data. Our approach is designed to tease out key ISS spaceflight environment effects relative to KSC Ground Control counterparts. Significant progress has been – and continues to be – made to integrate/compare the diverse multi-omics datasets to gain both new and comprehensive insights into the systems-level response(s) to the ISS spaceflight microgravity environment with the Arabidopsis genotypes. For example, pathway analysis was accomplished using targeted and untargeted approaches. The targeted approach involved extracting features from each dataset known to be associated with various structural (biochemical) pathways, and by comparing fold changes in ISS vs KSC Ground Controls across genotypes. The untargeted method involved the use of a LANL developed tool OPaver (Omics Pathway Viewer). OPaver enables us to map differential expression/abundance values onto curated Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway maps for transcriptomics, proteomics, and metabolomics datasets. Preliminary analysis indicates that some metabolic pathways were augmented by ISS spaceflight conditions vs KSC Ground Controls and are further being evaluated. Additionally, we conducted a targeted analysis of genes/proteins involved in chorismate/shikimate, flavonoid, and phenylpropanoid pathways. In general, we observed a global decrease in genes/proteins involved in the phenylpropanoid pathway for plants grown on ISS compared to KSC Ground Controls; this correlates with the observed decrease in measured lignin contents grown on ISS compared to KSC, corroborating the overall decrease in abundance of structurally related features across datasets in ISS samples.</p>
Bibliography Type:	Description: (Last Updated: 01/22/2025)
Abstracts for Journals and Proceedings	<p>Lewis NG. "Multi-omics analyses of ISS grown lignin-reduced Arabidopsis lines: New insights into potential of genetically modified vascular plants in the space environment." 37th Annual Meeting of the American Society for Gravitational and Space Research, Baltimore, MD, November 3-6, 2021.</p> <p>Abstracts. 37th Annual Meeting of the American Society for Gravitational and Space Research, Baltimore, MD, November 3-6, 2021. , Nov-2021</p>
Articles in Peer-reviewed Journals	<p>Hixson KK, Marques JV, Wendler JP, McDermott JE, Weitz KK, Clauss TR, Monroe ME, Moore RJ, Brown J, Lipton MS, Bell CJ, Pasa-Tolic L, Davin LB, Lewis NG. "New insights into lignification via network and multi-omics analyses of arogonate dehydratase knock-out mutants in Arabidopsis thaliana." Front Plant Sci. 2021 May 25;12:664250. https://doi.org/10.3389/fpls.2021.664250 ; PubMed PMID: 34113365; PubMed Central PMCID: PMC8185232 , May-2021</p>
Articles in Peer-reviewed Journals	<p>Overbey EG, Saravia-Butler AM, Zhang Z, Rath KS, Fogle H, da Silveira WA, Barker RJ, Bass JJ, Beheshti A, Berrios DC, Blaber EA, Cekanaviciute E, Costa HA, Davin LB, Fisch KM, Gebre SG, Geniza M, Gilbert R, Gilroy S, Hardiman G, Herranz R, Kidane YH, Kruse CPS, Lee MD, Liefeld T, Lewis NG, McDonald JT, Meller R, Mishra T, Perera IY, Ray S, Reinsch SS, Rosenthal SB, Strong M, Szewczyk NJ, Tahimic CGT, Taylor DM, Vandenbrink JP, Villacampa A, Weging S, Wolverton C, Wyatt SE, Zea L, Costes SV, Galazka JM. "NASA GeneLab RNA-Seq consensus pipeline: Standardized processing of short-read RNA-Seq data." iScience. 2021 Mar 26;24:102361. https://doi.org/10.1016/j.isci.2021.102361 ; PubMed PMID: 33870146; PubMed Central PMCID: PMC8044432 , Mar-2021</p>