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Project Title:	An Integrated Omics Guided Approach to Lignification and Gravitational Responses: The Final Frontier		
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Program/Discipline--Element/Subdiscipline:	SPACE BIOLOGY--Developmental biology		
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Task Description:

We propose a multi-omics study using the model plant Arabidopsis 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.
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 Arabidopsis 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.

Overall objectives:

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.

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.

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.

Rationale for HRP Directed Research:

Research Impact/Earth Benefits:

1). 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.
2). We are partnering with Ms. Kathy Lucchesi (K-7/8 teacher), at McCaffrey Middle School in Galt, California, and their largely Hispanic students on a program where elementary students can follow and repeat certain very safe plant growth experiments developed for the International Space Station (ISS) experiments, i.e., where we will assist on a “hands on” experience for the students, and their participation with the progress of our ISS multi-omics studies. This allows for the students to grow plants under similar conditions and to obtain information and insights on how the research impacts or benefits life on Earth. Our participation here includes offering instructions through Skype and written materials on the experiments at hand, and helping teach and inspire the young students about the joys and fun of the scientific method in experimental plant biology. Periodically, we will have these middle school students present results over Skype to our team meetings where such work is routinely evaluated.

3). Beginning September 2016, a high school student (Junior) has been involved in the project. He carried out/refined Arabidopsis growth conditions using the Science Carrier that will be fitted into the Advanced Plant Habitat, APH. The Washington State Space Grant Consortium is providing supplementary support for high school and undergraduate participation.

Task Progress:

Major emphasis (definition phase validation) upon initiation of the project has been to define the conditions required to obtain dependable, invariable, and reliable controlled growth of Arabidopsis plants using the given parameters and restrictions of the Advanced Plant Habitat (APH) prototype unit. This has been done in close cooperation/collaboration with the Payload Development Team (PDT) at Kennedy Space Center (KSC).
Approaches to the experimental design and implementation have thus focused on confirming the ability to successfully germinate and grow Arabidopsis for up to a 6-week harvest point in a reliable manner as previously done in other growth systems. This was required as a precedent to growing and harvesting the plants for experimental analysis, in order to prevent potentially confounding effects created by non-uniform environmental factors in the APH on ISS.

In conjunction with the KSC team, conditions were established to grow Arabidopsis in the newly developed APH, and which are considered potentially applicable for facile growth on ISS. This was developed based on numerous growth-out trials at Washington State University (WSU) over the last year to established best growth conditions, i.e., as regards water delivery, light intensity, humidity, etc.

The final growth out at KSC, which built up from the previous growth outs, was carried out into the APH Engineering

	Development Unit (EDU), and was initiated on January 10 and completed on February 21, 2017. Photographs were taken each day of the Arabidopsis growth-out. Metabolomics analyses were carried out on these various growth-out samples to ensure that results obtained matched our earlier findings for greenhouse/growth chamber grown plants. Studies also examined different times of metabolite extraction of plant lines that had been stored at room temperature up to 90 minutes; metabolite profile traces examined were unchanged during the different time frames. An Experiment Requirements Document (ERD) has been generated and will be submitted to NASA headquarters on March 8, 2017.
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