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Project Title:	An Integrated Omics Guided Approach to Lignification and Gravitational Responses: The Final Frontier		
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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) Starkenburg, Shawn Ph.D. (Los Alamos National Security)		
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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 have partnered with Ms. Kathy Lucchesi (K-7/8 teacher), McCaffrey Middle School in Galt, California, and their largely Hispanic students. Supplemental funding was provided to the school by NASA and the California Space Grant Consortium so that these middle school students can safely follow and repeat many of the plant growth and development protocols developed for the International Space Station (ISS) experiments. One purpose here is that the students grow plants under similar conditions and obtain information and insights on how the research impacts or benefits life on Earth and beyond (in the future).

Dr. Lewis (Washington State University) and Dr. Sato (NASA Ames) visited the school on September 28, 2017, to see first hand the work underway. Both visitors also gave talks on the project, as well as the broader ramifications of NASA research.

The Galt Herald wrote an article on this visit (http://www.galtheraldonline.com/), and students progress was further publicized through the NASA Space Biology Facebook (https://).

Progress with the students is also followed through regular Skype and/or FaceTime team meetings, with Drs. Lewis, Davin, and Costa (Washington State University-WSU). Dr. Hanson (University of New Mexico-UNM) also provided tutorials on photosynthesis and the use of a FluorPen for their studies.

Written materials on, and seeds for, the experiments at hand are also routinely provided by WSU. The additional aim here is in helping teach and inspire these young students about the joys and fun of the scientific method in experimental plant biology. Periodically, the middle school students present results to Dr. Lewis over where such work is routinely evaluated.

3). A Pullman High School student (PHS Senior Charles Pezeshki) has also been involved in the WSU 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 kindly provided supplementary support for both high school and undergraduate participation.

Mr. Pezeshki successfully completed his Senior Project, which led to several successful outcomes. He gave two posters (one at WSU for the University Showcase, and a second one in October 2017 in Seattle at the Annual Meeting of the American Society for Gravitational and Space Research (ASGSR) in a session for High School students nationwide). He was also supported by a travel grant from ASGSR to present his work.

Some of Mr. Pezeshki's activities included:

i). Sieving, washing, and autoclaving of Turface arcillite particles used as the growing substrate for plants on board ISS, as well as preparation of foam and gauze templates to support the seeds and plants in the final assembly of the Science Carrier unit.

ii). Recording and editing videos to present to NASA Kennedy Space Center (KSC) personnel, so that they could review our consortium's plant harvesting and processing methods used for RNA, protein, and metabolite extraction. These videos were instrumental for assessing constraints involved in foil pack dimensions required for plant samples for adequate preservation during storage and for rapid processing upon return to Earth. This was helpful for developing procedures necessary for optimizing astronaut crew time spent on harvesting and critical storage of samples in the limited freezer container space aboard ISS.

iii). Assistance with transgenic plant sample preparation for PCR gene screening analysis. This involved DNA extraction from freshly harvested plant leaves, and use of these samples as DNA templates for the PCR mixtures. The samples were amplified using a thermocycler instrument and then run on an agarose gel for qualitative analysis assessment.

iv). Developing protocols for harvesting and processing different parts of Arabidopsis plants, which included separating leaves, stems, siliques, flowers, etc., and then later on grinding tissues and preparing extracts suitable for our multi-omics studies. He continuously assisted in preparing plant tissue samples for metabolomic analysis. He also prepared a video presentation on this that was also very helpful for the NASA scientists/astronauts by recording this entire process beginning from harvest to extraction.

v). Studying effects of humidity on metabolite levels at different harvest times. Mr. Pezeshki individually processed leaf and stem tissues of four and six weeks old Arabidopsis wild type plants grown under low and high humidity conditions. He studied the growth and development (stem length and weight) of Arabidopsis wild type plants harvested at different time intervals. He also successfully completed this experiment without assistance. After he had expertise in preparing tissue samples suitable for metabolomics, he was assigned to help study the metabolomics of NASA-SVT (Science verification test) samples.

Task Progress:

In addition to testing, evaluating, and optimizing various Arabidopsis growth and development conditions at WSU and UNM, this reporting period focused on:
• Establishing and compiling the Experiment Requirements Document (ERD) for consideration and approval by the SLPS (Space Life and Physical Sciences) Program Executive for Space Biology at NASA Headquarters.

• Carrying out the required Science Verification Test (SVT) and Experiment Verification Test (EVT). This was in order to test conditions that will be employed on International Space Station (ISS).

• All tasks were done in conjunction with the Payload Development Team (PDT) at Kennedy Space Center (KSC), and completed on April 24, 2017 and September 04, 2017, respectively, and with our consortium team partners, David Hanson (University of New Mexico), Mary Lipton (Pacific Northwest National Laboratory-PNNL), and Richard Sayre and Shawn Starkenburg (Los Alamos National Laboratory-LANL).

1. Experiment Requirements Document (ERD)

In collaboration with the Payload Development Team, an Experiment Requirements Document (ERD) was prepared. On March 14, 2017, the ERD was presented to the SLPS Program Executive for Space Biology at NASA Headquarters who gave approval for the SVT to be started. After completion of the SVT, a revised version of the ERD was again presented to the SLPS Program Executive for Space Biology on 7/13/2017. Upon approval, the EVT was then initiated.

2. Science Verification Test (SVT)

The SVT was carried out in the prototype Advanced Plant Habitat (APH) Engineering Development Unit at KSC. APH is a plant habitat, capable of hosting multi-generational studies, in which environmental variables (e.g., temperature, relative humidity, carbon dioxide level, light intensity, root zone moisture content, and spectral quality) can be monitored, controlled, and data recorded. For grow out of wild type (WT) and transgenic Arabidopsis thaliana lines (48 plants in toto), seeds were placed in a Science Carrier. The Science Carrier included sensors, manifolds, and watering tubes and is divided into four quadrants. Each quadrant was filled with arcillite containing a slow release fertilizer.

Science Carrier Configuration and Plant Harvest:

• Five seeds each of A. thaliana WT, and transgenic adt5, adt4/5, adt3/4/5/6, WT/CCM, and adt3/4/5/6/CCM lines were adhered (using guar gum) to the gauze material in each of the 48 locations in the Science Carrier. Location of each line in each quadrant was randomly determined using a stratified randomized design. This random design was chosen so that there will be two plants of each line within each quadrant.

• The Science Carrier was next installed into the prototype APH Engineering Development Unit (EDU) and water was supplied to each of the four quadrants on March 15, 2017. Pictures were taken both from the side and the top of the APH and thereafter each day during the growth period.

• Fourteen days later (i.e., March 29, 2017), seedlings were thinned out so that only one remained in each spot. Prior to thinning Pulse-Amplitude Modulated (PAM) measurements were carried on all seedlings using a FluorPen.

• After four-weeks growth (April 12, 2017), the Arabidopsis plants looked healthy; some of them had started to bolt. PAM measurements were carried out on plants located in the front two rows of quadrants 1 and 4 with the FluorPen, and half of the plants in the Science Carrier were harvested.

• Because of the amount of plant debris accumulating after 5 weeks growth, it was decided to harvest the remaining plants in 2 groups: Quadrants 1 and 4 on April 21 (at 37 days) and quadrants 2 and 3 on April 24, 2017 (at 40 days).

• For both harvests, plants were collected from the left to right and from the front to the back of the Science Carrier, separated into stem and rosette leaf samples, transferred into labelled foil packets, weighed, and then frozen at –150°C using a conditioned cold block in a mini cold bag. Following harvest of the last plant, specimens were transferred into a –80°C freezer where they were kept frozen until shipped to Washington State University (WSU) on dry ice, where they were again stored at –80°C.

Lignin Analyses:

• In order to determine if the Arabidopsis plants grown in the APH for 37 and 40 days had attained the same level of maturity and lignification as those previously grown in our greenhouse at WSU, lignin compositions were estimated in a subset of plants. Thus, stems from WT and the mutant adt3/4/5/6 were freeze-dried, ground to a powder (in a mortar by means of a pestle), with cell wall residues next obtained as routinely performed in the Lewis laboratory. The cell wall residues of these selected plants were next subjected to thioacidolysis to estimate lignin contents. Lignin levels were significantly lower in stems from WT and adt3/4/5/6 plants grown in the APH for the SVT as compared to those grown in the greenhouse for a similar time period. This was due to the plants not having grown and developed to the same extent.

3. Experiment Verification Test (EVT), Run 1

The first EVT was carried out in the Advanced Plant Habitat (APH) Ground Unit (S/N001).

Science Carrier Configuration and Plant Harvest:

• Five seeds each of A. thaliana WT, and transgenic adt5, adt4/5, adt3/4/5/6, WT/CCM, and adt3/4/5/6/CCM lines were adhered (using guar gum) to the gauze material in each of the 48 locations in the Science Carrier. As for the SVT, the location of each line in each quadrant was randomly determined using a stratified randomized design.

• The Science Carrier was next installed into the prototype APH Engineering Development Unit (EDU) and water was supplied to each of the four quadrants on July 27, 2017. Pictures were taken both from the side and the top of the APH and thereafter each day during the growth period.

• Eleven days later (i.e., August 6, 2017), seedlings were thinned out so that only one remained in each spot. Prior to thinning Pulse-Amplitude Modulated (PAM) measurements were carried on all seedlings using a FluorPen.

• After four-weeks growth (August 23, 2017), the Arabidopsis plants had grown slower than during the SVT, none had started to bolt, and a few did not grow in Quadrant 1. Again PAM measurements were carried out on plants located in the front two rows of Quadrants 1 and 4 with the FluorPen, and half of the plants in the Science Carrier (24) were harvested.

• The remaining plants were harvested on September 04, 2017.

• As before, for both harvests, plants were collected from the left to right and from the front to the back of the Science Carrier, separated into stem and rosette leaf samples, transferred into labelled foil packets, weighed, and then frozen at –150°C using a conditioned cold block in a mini cold bag. Following harvest of the last plant, specimens were transferred into a –80°C freezer where they were kept frozen until shipped to Washington State University (WSU) on dry ice, where they were again stored at –80°C.

Lignin Analyses:

• In order to determine if the Arabidopsis plants grown in the APH for the EVT had attained the same level of maturity and lignification as those previously grown in the greenhouse at WSU, lignin compositions were estimated in a subset of plants. Thus, stems from WT, and mutants, adt5, adt4/5, and adt3/4/5/6 were freeze-dried, ground to a powder (in a mortar by means of a pestle), with cell wall residues next obtained as routinely performed in our laboratory. The cell wall residues of these selected plants were next subjected to thioacidolysis to estimate lignin amounts. Lignin levels were significantly lower in stems from WT and adt3/4/5/6 plants grown in the APH for the SVT as compared to those grown in the greenhouse. Again this was due to the plants not having grown and developed to the same extent as at WSU.

4. Experiment Verification Test (EVT), Run 2

A second EVT was initiated on January 31, 2018. Its purpose is to modify plant growth parameters in the APH in order to attain Arabidopsis maturity/lignin levels comparable to those obtained in the WSU greenhouse for all WT and mutant lines. This work is ongoing.

5. APH Flight Unit Readiness Status on ISS

• The APH Flight Unit was launched in two shipments to the ISS on OA-7 (April 18, 2017) and SpaceX-11 (June 3, 2017). The on-orbit installation was completed on October 27, 2017. The first power-up was successfully completed on November 27, 2017 followed by a 5-day functional checkout which was successfully completed on December 01, 2017.

• In order to further test the ISS APH unit, a Science Carrier with WT Arabidopsis seeds in Quadrants 2 and 3 and dwarf wheat in Quadrants 1 and 4 was shipped to ISS.

• A Plant Growth Test was initiated with Quadrants 2 and 3 (Arabidopsis seeds) and Quadrants 1 and 4 (wheat seeds) successfully flood filled on January 22, 2018, and February 8, 2018, respectively.

• All specimens appeared to be growing well to date (as of February 28, 2018).

6. Sample Preparation for Metabolomics, Transcriptomics and Proteomics Analyses.

The overall scope of this proposal is to carry out transcriptomics, proteomics, and metabolomics analyses on leaves/stems of all plants harvested after 4 and 6 weeks of growth in the APH. Because the amounts of tissues

recovered might be limited (~200 mg), we tested:

• An extraction method developed by PNNL allowing metabolites and proteins to be extracted from the same tissue (~150 mg) with these used for subsequent metabolomics and proteomics analyses. Twenty-four proteins samples were sent to PNNL for QC analyses: All passed. The corresponding metabolites samples were analyzed at WSU and also passed the QC test.

• An extraction method using ~50 mg tissue (fresh weight) to isolate mRNA for transcriptomics analyses. Forty mRNA samples were sent to LANL for QC analyses. All passed the QC analyses.

7. Seed Viability

In addition to the additional genetic transformations and establishment of requisite growth conditions etc. for the Arabidopsis, seed viability tests were carried out at WSU. These were studied because of the long lag time between shipping the two Science Carriers to ISS and the much later initiation of the experiments by flood filling the four quadrants.

Seed viability testing at WSU included:

• A Science Carrier prepared as before, with WT Arabidopsis seeds in the four quadrants.

• The first quadrant was flood filled, with all seeds germinating. After 2 weeks, the seedlings were thinned. All plants grew well the throughout the 6 week time period.

• The second, third, and fourth quadrants were each flood filled at a 6 week interval. As for Quadrant 1, all plants grew well.

• There were no problems noted with seed viability testing in the Science Carrier for at least 24 weeks.

Bibliography Type:	Description: (Last Updated: 01/22/2025)
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