**Research Campaign: Space Biology Reference Experiment Campaigns for High Fidelity Plant Physiology**

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**I. Introduction:** The NASA GeneLab Plant Analysis Working Group (AWG) is an international consortium of plant researchers with expertise in spaceflight experimentation and in state-of-the-art genomic, transcriptomic, proteomic, metabolomic and epigenomic analyses. The members have regularly met to discuss the needs and goals of plant spaceflight research over the last three years and the following recommendations have emerged from these discussions.

For context, plants will be critical to the future of human space exploration. Plants will likely be the foundation of a bioregenerative life support strategy for sustainable, deep exploration and habitation of space by humans. Plants will provide nutrition for long-duration missions and have the potential for the production of critical nutraceuticals (Douglas et al., 2021) and, with appropriate genetic modifications, the capacity to produce other advanced bioproducts such as pharmaceuticals. Algae are also important for their nutritional value, as well as for life support revitalization schemes and closed loop bioproduction systems.

**II. Reference Experiments:** The GeneLab Plant AWG specifically recommends conducting a series of Space Biology Reference Experiment Campaigns in the coming decade. In parallel, we recommend the adoption of the basic principles of Open Science/Open Research (Porterfield et al., 2021) as recently outlined by the *National Academies of Sciences, Engineering, and Medicine* (https://doi.org/10.17226/25116). Our current knowledge of plant spaceflight responses is drawn from a disparate series of experiments performed by a wide range of experimenters, each pursuing a particular hypothesis or goal. This kind of analysis will remain important, but a complementary systematic research approach is needed to make even more rapid progress in our understanding of plant spaceflight responses in order to deliver the critical science required to engineer for the coming near-term exploration opportunities. This would be through a series of well-replicated studies of some carefully chosen model plants and crops where the latest analytical approaches, including omics-level analyses, could be employed to provide a comprehensive view of how these plants respond to growing in space.

The plants used, and design of these reference experiments, would need to be defined by the research community and other spaceflight stakeholders, to cover multiple model systems and critically, to encompass the whole life cycle(s) of the target plants. The aim of these experiments would be to rapidly release data for community-wide analyses and so help provide some landmarks throughout the lifecycle of how various plants grow in space. This would be the data upon which researchers could build new hypotheses, develop ground-based testing and support future space exploration including approaches such as genetically adapting crops (Haveman et al., 2021) to thrive in extra-terrestrial environments. Funding for ground-based data-driven translational research from these reference data sets would also create a dynamic ecosystem of funding and support for innovation, education, outreach, and knowledge transformation.

In addition, even more rapid progress could be made by combining the varied expertise of multiple research groups towards achieving a focused goal, in this case the implementation and then broad analyses of reference experiments. Thus, periodic calls for multi-omics reference experiments, defined as collaboration between various members of the spaceflight research community to follow responses in major model species, would help develop these nimble, integrated teams and prompt transformative leaps in our understanding of plants in spaceflight. Providing mechanisms for the participation of partners from ESA, JAXA, or other agencies as integral members of such teams would also greatly enhance progress toward space research milestones, as would leveraging the synergies possible through partnerships with other federal agencies such as NSF, USDA, and DOE.

The specific details for each reference campaign, such as target species, beyond stressing the need for multi-generational, multi-replicated designs, will not be discussed herein as these should be the subject of broad community and stakeholder input. Instead, below we will discuss some of the basic scientific research needs for the discipline and propose best practices for the research design and data that would be generated by these efforts. These factors are critical to ensure both the production of robust datasets and the future interoperability of the results for as broad a range of space-biology research as possible.

**III. Develop a robust set of ground-based analogs:** Validation of spaceflight analogs that simulate the spectrum of spaceflight environments from Low Earth Orbit (LEO) to lunar and beyond is a critical goal for the next decade to help supplement the still likely rare opportunities for true spaceflight experimentation (Chin et al., 2021). This emphasis should extend beyond simulated microgravity and altered gravity loads using equipment such as clinostats, random positioning machines, rotating wall vessels and magnetic levitators, to include other aspects of spaceflight such as the altered circadian periods and increased exposure to solar and galactic cosmic radiation that accompanies spaceflight. The validation of spaceflight analogs can by no means replace the essential nature of true spaceflight studies and the continued support and expansion of research capabilities in true spaceflight will remain the cornerstone for understanding extraterrestrial impacts on plant life. However, well-characterized analogs will add enormous value to insights from the likely rare spaceflight opportunities. A robust ground analog research program should be a hallmark of a healthy space-flight program. Results from well-designed spaceflight reference experiments would in turn help provide landmarks for the validation of current and future analogs.

**IV. Expand the diversity of plant species studied in spaceflight:** Defining appropriate models for reference analysis in space will require broad input from the research community and other stakeholders but should be made with careful consideration of existing genetic tools (such as the quality of genome assemblies and annotations). Tables 1 and 2 contain a brief listing of some potential target species. Experiments aimed at studying model plant species throughout their life cycles (e.g., seed-to-seed, multigenerational studies) will yield fundamental insights into plant spaceflight responses that in turn can inform the development of crop plants and drive practical applications of plants in bioregenerative life support systems. However, the broader the set of plants that are used in these experiments, the more robust will be the inferences drawn from their common reactions to the spaceflight environment. This is an important justification for a reference campaign. The more plant species analyzed within the theme of a multigenerational, well-replicated design where the data is released as quickly as possible for community analyses, the more we will understand about what it truly means to grow in space.

**V. Enhance the impact of previous scientific investments:** We recommend creating opportunities for specific, smaller NASA Research Announcements and post-doctoral fellowship opportunities focused on mining and analyzing previously deposited spaceflight data. The focus should be on capitalizing on the translational potential for these data to create new working knowledge for future operations. The data output of reference campaigns has the potential to provide significant enhancement to research objectives for minimal investment. Additionally, enhanced analyses of previous spaceflight missions with special consideration of those that incorporate data from multiple GeneLab GLDS/experiments and/or multi-omics experiments in the context of relevant reference experiment data, will be particularly impactful. Such add-on studies would also be well-suited to postdoctoral fellowships, thus providing an investment in future leaders in the space biology field and promoting omics-level literacy in this important tier of researchers.

**VI. Microbiome impacts on biological systems in spaceflight environments:** An appreciation of the importance of interactions of microbes with themselves and with other organisms such as plants is recognized as a key advance in our understanding of life on Earth. Extending this insight to the spaceflight environment will be a critical goal for the coming decade. NASA has made important steps forward in initiating research into how microbes respond to spaceflight and in relation to plant biology, this program should be extended to ask how microbes affect plants and how plants affect microbial communities in the spaceflight environment. Plant- microbe interactions would be a logical element of a reference campaign but again, the precise interactions to be studied should be the subject of community and stakeholder discussions based on the current state of the art at the time of implementation.

**VII. Broader impacts of reference experiment campaigns**: The campaigns proposed herein will have broad impact beyond just providing fundamental insight into how plants respond to space.

1. The importance of the reference experiment data will prompt the key discussion about the metadata that should be provided to make these experiments useful into the future. Setting some minimal metadata requirements for each spaceflight experiment is essential but will only be sustainable if these requirements are agreed upon by the community, much as the MIAME standards helped the databases populated by microarray data to mature into the critical transcriptomics resources they are today. Table 3 lists some plant specific metadata for consideration.
2. Identifying specific cultural or hardware counter-measures to increase crop reliability in space-biology related environments.
3. Overarching framework for optimizing various crops for space flight production.
4. Create biobanking to preserve samples for future analytical technology. This would allow NASA to “re-run” these experiments later with the most recent analytics.
5. The data from well replicated reference experiments would drive the training of machine learning models and applications.
6. Develop standards and infrastructure for expansion into range of future plant/crop studies for adoption on long-duration flights, lunar and Martian surface.
7. Prompting retrofitting of existing plant growth infrastructure and/or testing and/or deployment in new space cropping systems.

**Table 1: Models and crops of interest for campaign experiments**

| **Species** | **Specific Utility/ Characteristics** | **Existing tools/ Advantages** |
|---|---|---|
| *Arabidopsis thaliana* | Understanding the genetic and molecular response of the space biology relevant environment. | Essential reference tool for early validation of genetic modification approaches and new flight hardware. |
| A plethora of microgreen candidates: Radish, mizuna, chia, kale, amaranth, sunflower, pine nuts, etc. | High-value for astronaut psychological well-being. Nutritional and fiber benefits with only moderate wasted biomass. | Fast growth, low usage of area, and low necessity of nutrients, source of beneficial plant compounds (antioxidants, enzymes, etc.). |
| Leafy greens (cress; e.g. *Barbarea verna*; *Lepidium sativum*), | High flexibility for incorporation into broadly palatable end-products. Nutritional and fiber benefits with only moderate wasted biomass. | Acceleration of crop cycling with environment optimization and variety selection. Great use as an immediate diet supplementation, physiological support for the crew. |
| Legumes (sweet pea, and bean landraces) | High nutrient density with multiple harvests per generation. Well-rounded nutrient profile to provide a more balanced diet. | Strong literature basis for microbial inoculation. N-fixation will be of use on the M oon and Mars. |
| Algae, aquatic plants such as Wolfinia, Londoltia and Lemna | Little to no wasted biomass, food and high-value bioproduction capable. Useful in closed-loop bioregenerative systems | Generational mutation data already acquired in model species. Variable strains/species allow optimal tolerance/attribute selection. |

Optimization of specific terrestrial agriculture standards among crops (Table 2) will also be essential for extraterrestrial agricultural objectives to be realized. Lessons from these species should be prioritized for preliminary validation but, additionally, other high value crops (Table 3) should be evaluated for molecular and agricultural endpoints on varied substrates (lunar, Martian, solid/liquid waste stream). The basis for further development/optimization of these crop species should be delivered upon completion of the research campaign.

**Table 2: Long term crops for consideration**

| Species | Specific Utility/Characteristics | Improvement challenges |
|---|---|---|
| Tuber crops (beets, potato, sweet potato, yam) | Likely "best in class" for nutritional profile. Some species allow little to no wasted biomass (e.g., beet greens and root tuber). | Many crops have significant need for preservation. Time to yield and bioproduction off-Earth will provide significant enhancements to total yield. |
| Cereals (wheat, barley, rice, etc.) | Conversion of terrestrial large-scale food production practices off earth will likely incorporate perennial cereal crops. Readily processed to "shelf stable" food supplies. | High land mass, and volumetric requirements for large-scale growth. Volume reduction (e.g., dwarf varieties) and other space optimizations (e.g., improved harvest index). |

**Table 3: Plant Reference Experiment Campaign, Phenomics and Advanced Metadata**

| Plant sensing | Environment sensing | Concepts to consider |
|---|---|---|
| Throughout the life cycle (including bolting) | Light intensity and wavelengths | Time series analysis (3, 8, 12 days) |
| Imaging reflectance spectrum | Real time nutrient status and plant uptake / use efficiency | Spatial resolution |
| Analyzing crop morphology, roots and shoots | Humidity (soil and atmospheric) | Repeat the same experiment at least 4 times (4 missions) |
| Crop ion content | Rootzone $H_2O/O_2$ | Consistent use of a WT reference organism |
| Crop nutrient content | pH monitoring of rootzone | Yield |
| Photosynthesis rates | Vapor pressure deficit control | Harvest index |
| PAM Fluorescence | Atmospheric $O_2$, $CO_2$ | Water use efficiency (WUE) |
| Spatially resolved Spectroscopy: e.g., Raman, FTIR | Gravity-sensing | Total mineral nutritional status analysis (macro/micro nutrients) |
| Leaf (phyto) microbiomes | Soil rhizosphere microbiome | Microbiome community |

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