**Growing Plants Under Altered Gravity**

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**Introduction**

Growing plants in space offers the possibility of sustaining astronauts on extended missions without the need for extensive resupply of the elements required for a human life support system. Plants could supply oxygen, food, bioremediation systems, fuel, organic matter as well as improve the psychological well-being of the crew. One of the biggest undefined challenges in growing plants in both spaceflight and in future planetary bases is the altered gravity environments that will be imposed in these situations. Altered gravity will affect physical features such as buoyancy, convection and sedimentation that will impact plant function. However, to date there is little data allowing researchers to understand how plants will respond to these conditions and thus, how to develop necessary genetic- or engineering-based countermeasures.

**Goals, Objectives, and Investigations**

***Goal 1: Understand how plants grow in altered gravity environments***

Plants do grow in space, albeit with some modifications relative to equivalent plants on Earth such as developing to a smaller size, roots typically grow skewed with more waving and have less root hairs (Kordyum et al., 2021). These plants also often exhibit a suite of other changes such as reduced photosynthesis, changes in metabolism and secondary metabolite production and reduced lignin. How far these changes are driven by the effects of reduced gravity remains poorly defined. Plants grown in space and the Moon and Mars will be growing outside the Earth’s magnetic field and will also experience increased radiation exposure, hence it is an important consideration to tease apart the effect of altered gravity on plant growth and development from these other potential stresses. This goal can be achieved by combining:

1. an extensive ground-based research program built around exploring plant responses to a continuum of gravity levels. Success will require investment in improved microgravity analogs and plant growth centrifuges coupled with easy access to these capabilities and clear community standards for their use.
2. spaceflight experimentation to explore true microgravity and partial gravity effects in the context of the other features of this environment. Critical here will be investment in improved flight plant growth capabilities and especially new data collection and sensor systems to allow for more comprehensive remote sensing of plant responses in real time with reduced requirements for sample return.

Objective 1: Defining altered gravity growth environment

Investigate how plant physiology, growth, and development change in a continuum of gravity levels using a comprehensive ground-based program.

**Priority 1**: Building standard gravity altering growth instruments and nomenclature

Although access to sustained microgravity via spaceflight remains the gold standard for conducting microgravity and reduced gravity experimentation, access to this resource remains highly limited. Therefore, researchers heavily rely on ground-based analogs. Currently, microgravity is simulated using clinostats, random positioning machines (RPMs), magnetic levitation devices, drop towers and hyperbolic flights (Kiss et al., 2019). However, the main disadvantage to these instruments is the lack of standardization in instrumental design that affects experimental reproducibility and comparability between groups of researchers. Instrument design hugely influences the size, stage and type of plants that can be analyzed. In addition, there are currently no clear community developed guidelines regarding optimal clinostat and random positioning machines settings. For example, clinostat users typically distinguish between slow rotation as 1-4 revolutions per minute (rpm) and fast rotation as 50-120 rpm. Although rpm is used to describe the rotation speed for both clinostats and random positioning machines, it does not measure the forces experienced by the sample during rotation.

By contrast, hypergravity can be simulated with centrifuges. For small-scale experiments such as on seedlings, the 3-D clinostat such as Gravite can be programmed for both microgravity and hypergravity by varying rotation axis, whereas large scale experiments require large diameter centrifuges such as the one available at European Space Research and Technology Center.

Recommendations and impacts:

1. Implementation of a large 3-D clinostat that includes both microgravity and hypergravity capabilities. The clinostat should be adjusted to include the capability to switch between microgravity (clinostat/RPM) and hypergravity (centrifuge) mode and include programmable rotational capabilities to simulate partial G environments in the Moon and Mars. It should be sized around a typical walk-in growth chamber, with a growth area of up to 80.9ft² and growth height of up to 95 inches. The large clinostat should have control parameters for light, temperature, atmospheric gas composition, humidity, and radiation.

*Impact: Such a clinostat will provide larger experimental space for researchers to include larger plants, more samples and longer growth duration. Researchers will have additional options to include experiments that include both hyper and microgravity effects. Being able to grow large plants with a high degree of replication remains a current severe limitation on advancing our knowledge of how reduced gravity environments affect plants, especially in the context of larger plants needed for possible bio-regenerative life support systems.*

1. Comparison of microgravity simulators, between clinostats and RPMs with real microgravity and reduced gravity environments. As clinostats and RPMs are the most accessible microgravity simulators available, particular focus on the design of these instruments will be the most beneficial for researchers. Publications of data comparing the results of samples grown under the influence of real microgravity at the International Space Station (ISS) with selected designs of clinostats and RPMs under highly controlled environments should be a priority to inform future studies.

*Impact: Results obtained from microgravity simulators that best mimic real microgravity can be used to predict physiology and development changes that are heavily influenced by gravity.*

1. Development of guidelines for the use and design of gravity altering growth instruments. Guidelines should include best practices for describing the use of gravity altering growth instruments, measurements of gravitational force in these instruments, essential design patterns, rotation algorithms and quality control for instrument reliability and consistency across different manufacturers, and standard definitions.

*Impact: Increased experimental reproducibility within and between laboratories will better support formal and informal research teams and collaborations to define relationship between gravity modification with sample growth, physiology, and development.*

***Goal 2: Improve plant growth hardware to increase their size and usability to allow production of a wider range of plants and facilitate rigorous experiments at fractional Gs in multiple environments***

The ISS provides a unique microgravity environment that is difficult to recreate on Earth. Nonetheless, the main limitations in growing plants at the ISS are the size constraints of growth hardware and secondary growth artefacts caused by poor aeration and convection. This in turns affects: 1) the plant species that can be grown, 2) the growth and development of the samples, 3) the developmental stages of which the plant can be grown, 4) phenotype measurements, and 5) growth duration. One of the key points in understanding how plants cope with gravitational changes is to accurately track changes that occur throughout their lifetime and if possible, include multi-generational data from its offspring. Therefore, developing robust phenotyping systems that include upgrades in growth hardware for robust and uniform plant growth in multiple environments, imaging facilities, remote sensor technologies and metabolomic instruments are required to address this key issue.

Objective 1: Upgrading plant growth hardware to expand phenotyping capabilities in different environments

Improving the design and output of currently available plant growth hardware to enable research into different types of plants of varying sizes and developmental stages. In addition, plant growth hardware can be made versatile and modular to fit in different environments, particularly spacecraft and space stations either on the Moon or Mars for uniform plant growth. This will be crucial in providing comparable plant growth data across different environments.

**Priority 1**: Building larger plant growth hardware for spaceflight with artificial gravity platform. APH is the most sophisticated hardware that is equipped with full automation and measurement of light, gas exchange, humidity, photoperiod, temperature, and root zone moisture. Despite being largest plant growth hardware, its size still limits the type of plants and their growth duration. Considerations for new growth hardware for the purpose of long-haul space exploration in the Moon and Mars should include the automation capabilities of APH along with larger growth chambers that can be for longer duration and can be transplanted between ISS and rocket modules and space stations in Mars and the Moon. In addition, the upgraded growth hardware should include a Centrifuge-equipped Biological Experimental Facility (CBEF) (Shiba et al., 2017) for artificial g environment in microgravity space environments.

Recommendations and impacts:

1. Larger growth chamber than APH provides more space to include more plants in the experiments, or the selection of larger plants, and/or the capability to grow plants into maturation and possibly multiple generations.

*Impact: The inclusion of more plant species to diversify edible and multipurpose plants that can be grown in microgravity for space exploration. This also enables the study of how plants interact with close by plants when grown next to each other.*

1. Increase plant growth duration on growth hardware for long term plant growth that encompasses reproductive stage(s) and multi-generation.

*Impact: Enables the studies into mature and reproductive stages of plant growth, and multiple generation. This will be instrumental in understanding how the gravity factor affects fruit or seed yield, plant fertility and trans-generational epigenetics.*

1. Development of a CBEF platform in plant growth hardware for fractional g studies. Currently, all control 1g experiments that are based on ground control are not fully comparable with the space environment because the space environment differs in cosmic radiation, microbial environment, lack of convection and potential launch and return effects such as vibration.

*Impact: Researchers can generate high quality and robust data that determines the main influence of fractional gravity on plant growth and development, whilst excluding effects of the space environment. This can translate to higher quality research and associated publications.*

**Priority 2**: Expanding phenotyping capacity of plant growth hardware

The most sophisticated growth hardware on the ISS, APH, is well equipped with three cameras (Monje et al., 2020) to collect images to phenotype aerial parts of the plant. Nonetheless, processes that occur below ground have been largely neglected because there has been no high throughput or reliable phenotyping capabilities for roots. Root phenotyping is presently limited to astronauts taking photos of plants grown in agar setups either in Petri dishes or Magenta GA-7 vessels that display the whole root system. This in turn, requires researchers to manually stitch together 2-D composite photos of the root system, which typically results in low resolution of root spatial architecture information. Being able to accurately phenotype 3-D spatial architecture is essential in understanding how microgravity affects the dynamics of gravity perception and response, circumnutation and growth of roots and shoots. As such, high throughput phenotyping technologies, particularly with telemetry advantages for remote access at e.g., the ISS, or future bases on the Moon or Mars need to be developed.

Recommendations and impacts:

1. Explore the potential use of non-invasive 3-D phenotyping such as using magnetic resonance imaging (MRI), and X-ray computed tomography (X-ray CT) to accurately track plant growth and development over time in space.

*Impact: Non-invasive phenotyping that does not disturb the living root and aerial system allows repeated sampling over the duration of plant growth to monitor dynamic phenotypes over time (Atkinson et al., 2019). The current state-of-the-art in this area in the ground is MRI and X-ray CT sampling that collects information about 3-D spatial plant architecture, as well as internal plant anatomy such as the xylem vessel and phloem (Piovesan, et al., 2021).*

1. Using the Spectrum imaging system for non-destructive 2-D phenotyping. Spectrum is a multi-spectral imaging system designed for fluorescence imaging at the ISS. It is fitted with LED grow lights and an imaging system that can take both bright field and fluorescence data. Spectrum has a multifunctional carousel that can fit four 100 mm x 100 mm Petri plates which makes it suitable for imaging seedling stages of different plant species. As such, plants can be grown in Petri dishes in Spectrum for a short period of time with images automatically taken by the machine at set time points or be used as an imaging platform by astronauts for plants grown in Petri dishes. The latter is advantageous for astronauts to take consistent photos with minimal handling and training, whilst enabling researchers to view their experiments on the ground almost immediately.

*Impact: Spectrum can be easily adapted as a 2-D plant phenotyping platform for plants grown in Petri dishes at the ISS with higher throughput than manual photographing. Researchers have the benefit of viewing their plants during experimentation at the ISS and have consistent image data that will not require much post-processing.*

**Priority 3**: New tools for quantification of metabolomics and small molecules. The use of metabolomics is still under-utilized despite its potential in revealing changes that occur in pathway metabolism and its ability to measure phenotype via metabolites and small molecules. Furthermore, very little is known on how space environment affects plant volatile emission and root exudation. To collect VOCs, the growth chamber can be designed with an air sealed chamber with a fresh air inlet and an air outlet that is fitted with an adsorbent volatile trap. Alternatively, VOCs released on aerial parts of individual plants can be encapsulated by solid phase microextraction fibers in tubes and then be stored in an airtight container for GCMS analysis on ground. For root exudate collection, plants that are grown in hydroponic systems such as the Passive Orbital Nutrient Delivery System, PONDS can have their solution harvested at intervals and stored cold.

*Impact: Researchers can integrate metabolomics in their multi-omics studies, especially since the proposed metabolite collection methods are non-destructive. New literature on the effect of altered gravity on plant emission of VOCs and root exudates.*

In summary, the study of plant growth in altered gravity requires ground-based microgravity simulators to mimic the microgravity environment experienced by plants grown in space. Simultaneously, this should be complemented with studies of plants grown in space with fractional g for comparison. Omics that include transcriptomics, proteomics and metabolomics with accurate growth phenotype data based on the use of sensor and analytical technologies will provide the best description of plant developmental changes that occur at altered gravity.

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