National Aeronautics and Space Administration

A Researcher's Guide to:

INTERNATIONAL SPACE STATION

Plant Science

This International Space Station (ISS) Researcher's Guide is published by the NASA ISS Research Integration Office.

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Cover and back cover:

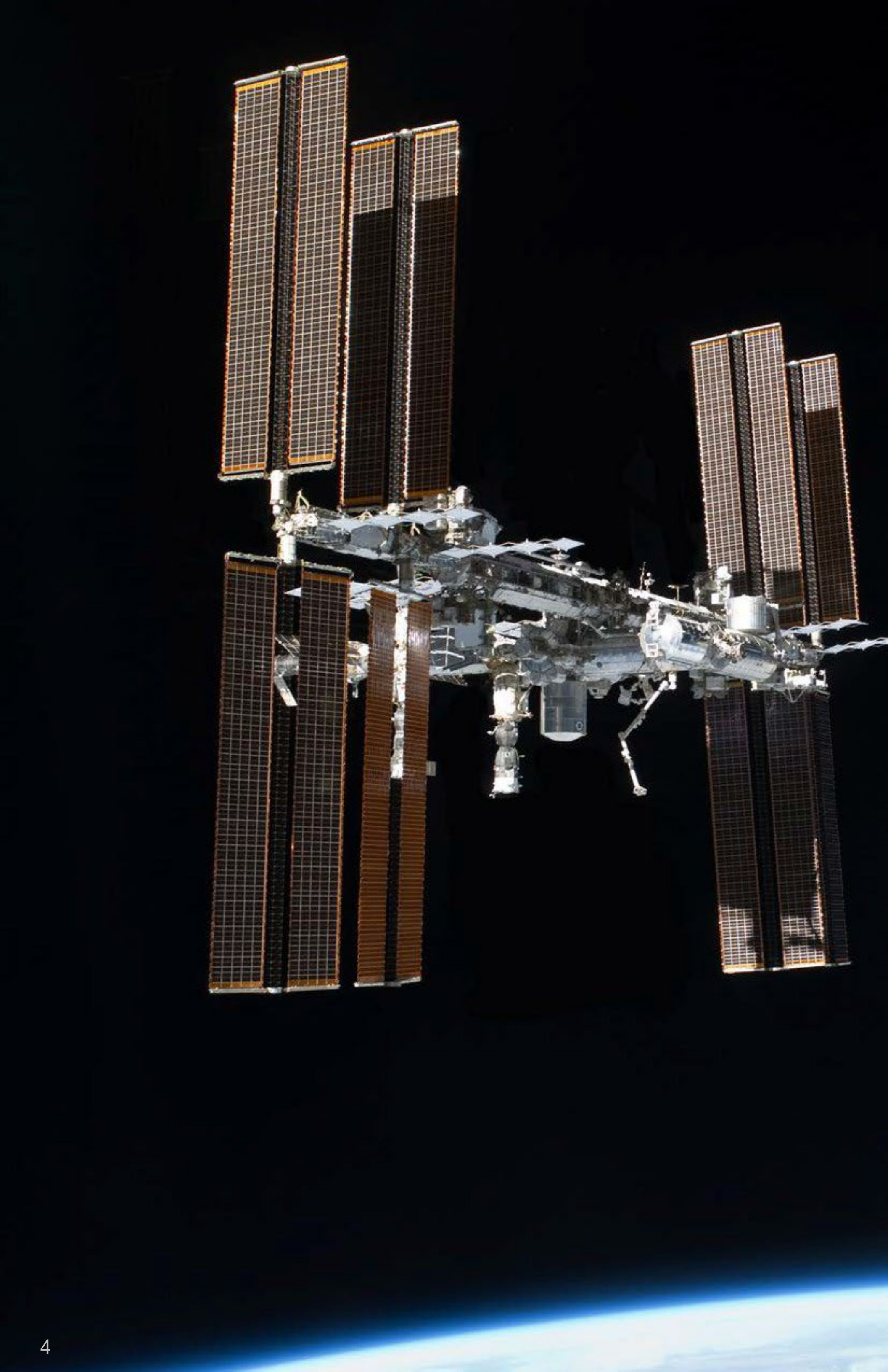
a. *NASA astronaut and Crew-3 member Tom Marshburn looks at chiles growing inside of the Advanced Plant Habitat. Crew-3 performed the second harvest of chiles aboard the ISS for the Plant Habitat-04 experiment. This plant experiment, one of the station's most complex to date because of the long germination and growing times added to NASA's knowledge of growing food crops for long-duration space missions. (Source: NASA)*

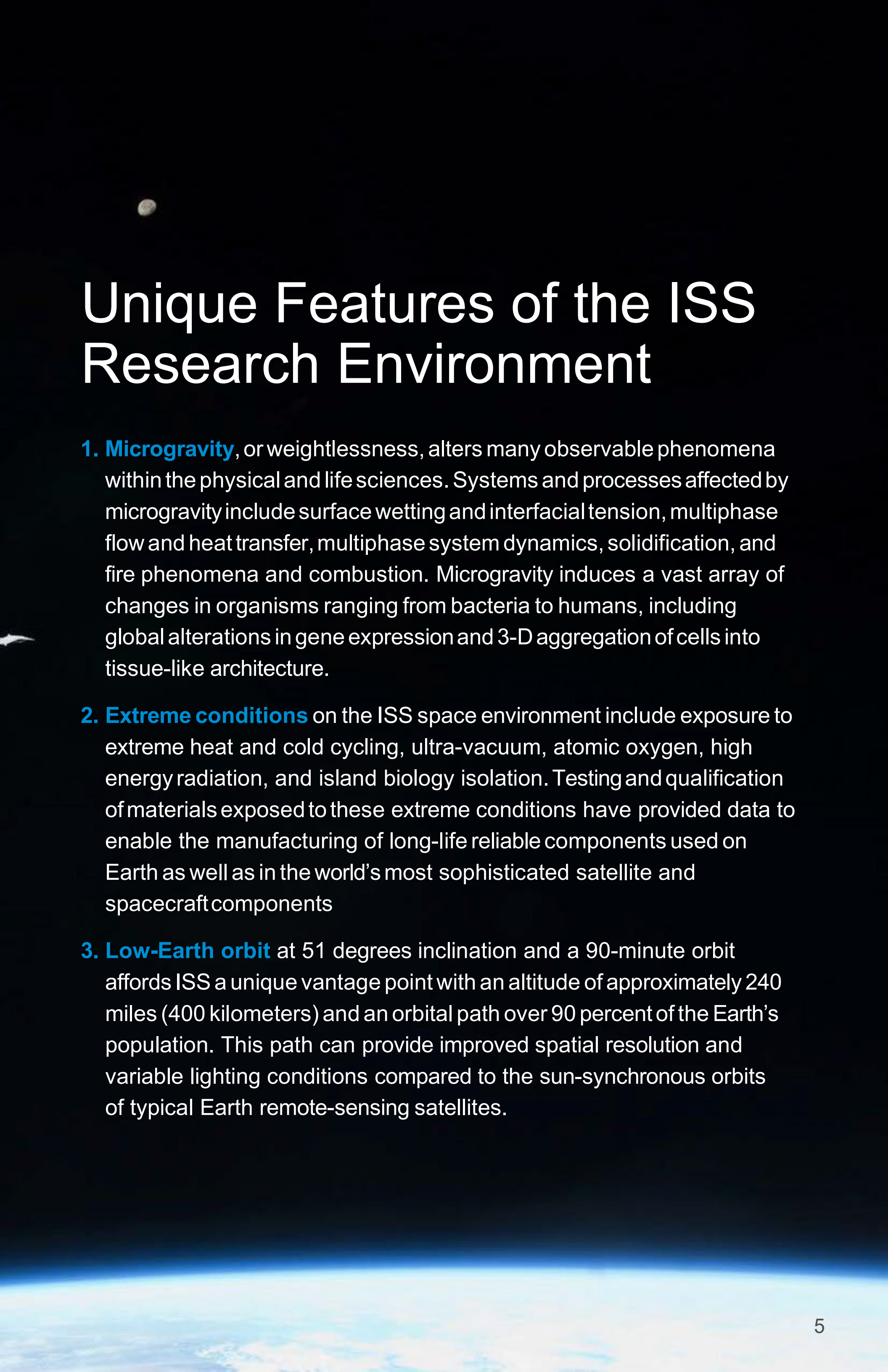
a. Back Cover:
Top: Water droplet on pea leaf grown in Lada chamber on ISS. The lack of buoyancy-driven convection in microgravity alters the behavior of fluids and gases at the leaf interface. (Source: NASA)
Bottom: Amara Mustard plants in Small Plant Pillows on day 41 of the Veg-03L experiment. (Source: NASA)

The Lab is Open

Soaring 250 miles above Earth, the International Space Station (ISS) is a modern wonder of the world, combining the efforts of 15 countries and thousands of scientists, engineers, and technicians. The ISS is a magnificent platform and laboratory for all kinds of research to improve life on Earth, enable future space exploration, and understand the universe. This guide is intended to help potential researchers plan and carry out plant experiments aboard the ISS, provide an overview of plant growth chambers available for use, and discuss the integrated spaceflight environment. This includes using the continuous freefall of the microgravity environment to study the role of gravity and other spaceflight environment effects on plant growth and metabolism in low Earth Orbit.

Cosmonaut Gennady Padalka harvests radishes from the Lada Plant Chamber (June 22, 2009).





Unique Features of the ISS Research Environment

1. **Microgravity**, or weightlessness, alters many observable phenomena within the physical and life sciences. Systems and processes affected by microgravity include surface wetting and interfacial tension, multiphase flow and heat transfer, multiphase system dynamics, solidification, and fire phenomena and combustion. Microgravity induces a vast array of changes in organisms ranging from bacteria to humans, including global alterations in gene expression and 3-D aggregation of cells into tissue-like architecture.

2. **Extreme conditions** on the ISS space environment include exposure to extreme heat and cold cycling, ultra-vacuum, atomic oxygen, high energy radiation, and island biology isolation. Testing and qualification of materials exposed to these extreme conditions have provided data to enable the manufacturing of long-life reliable components used on Earth as well as in the world's most sophisticated satellite and spacecraft components

3. **Low-Earth orbit** at 51 degrees inclination and a 90-minute orbit affords ISS a unique vantage point with an altitude of approximately 240 miles (400 kilometers) and an orbital path over 90 percent of the Earth's population. This path can provide improved spatial resolution and variable lighting conditions compared to the sun-synchronous orbits of typical Earth remote-sensing satellites.

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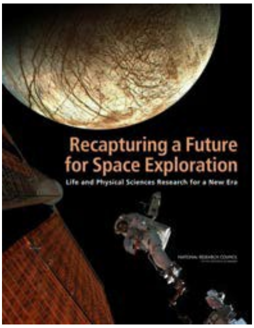
Why Use the ISS as a Laboratory?

The ISS provides a unique platform from which to understand how plants respond to gravity and the spaceflight environment. This knowledge is important because plants and their microbial communities are critical components of bioregenerative life support systems (BLSS) that will enable humans to thrive in space.



The National Research Council's 2011 Decadal Survey Report, "Recapturing a Future for Space Exploration: Life and Physical Sciences Research for a NewEra," (http://www.nap.edu/catalog/13048.html) recognized the significance of understanding plant responses in microgravity for both space exploration and terrestrial applications. A new report schedule for release in the summer of 2023 will have amended recommendations.

The 2011 report had a number of recommendations for plant research, including the following:

1. The establishment of a robust spaceflight program of research analyzing plant and microbial growth and physiological responses to multiple stimuli encountered in the spaceflight environments.

2. The establishment of a research program aimed at demonstrating the roles of microbial-plant systems in long-term life support systems.

A number of knowledge gaps exist that are well-suited to be addressed using the current capabilities of spaceflight hardware and analytical capabilities on the ISS. These gaps point to the necessity of maximizing the science return from each spaceflight opportunity and the need for both basic and applied research as well as transcriptomic, proteomic, and metabolomic analysis in model plants. These studies as well as life cycle research are necessary for developing effective horticultural techniques and validating environmental conditions to enable the incorporation of plants in BLSS. A partial listing of these research questions is provided in Table 1.

Table 1. Partial List of Research Questions

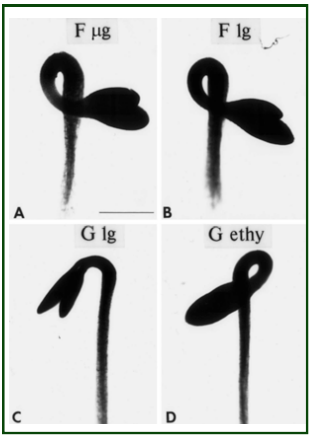
Topic Area	Potential Research Questions Suitable for ISS Investigations
Gravity Sensing	• What are the primary gravity receptors in leaf stem and root tissue? • What are intermediate signals at transcriptional, biomolecular. and physiological levels? • What interactions with thigmotrophic, phototrophic, and hydrotropic stimuli occur, and how are they differentiated?
Plant Physiology	• How do light and gravity responses interact? • Does the spaceflight environment induce stresses (μ-gravity, elevated CO_2, diffusion limited chemical exchange, root zone hypoxia, etc.), and what are primary signals and hormonal changes affecting development? • What are the effects of spaceflight environments on primary physiology: photosynthesis, respiration, transpiration, nutrition, and secondary metabolism? • How do the interactions of multiple environmental stimuli affect productivity and bioavailability of bioactive products? • How does the gravity effect on structural carbohydrates manifest itself over multiple generations? • Does microgravity affect genetic stability?
Plant/ Microbe Interactions	• What aspects of the spaceflight environment regulate resistant and/or susceptibility to plant pathogen infection? • Are there gravity effects on virulence and if so, are they species/strain specific? • How does the spaceflight environment affect the development of beneficial plant/microbe associations?
Life Support Systems	• How can horticultural approaches to sustained production of edible crops be implemented? • What are the effects of environmental stresses (lighting, CO_2, root zone moisture, O_2, trace gases) on productivity? • Are models of crop productivity developed for terrestrial conditions valid in spaceflight environments? • How do plants and microbes interact with physical and chemical life support systems?

LESSONS LEARNED FROM PLANT RESEARCH ON ISS

INSIGHTS INTO FUNDAMENTAL PLANT BIOLOGICAL PROCESSES

Interactions Between Gravity and Other Space Environmental Stimuli

Gravity has profound effects on the growth and development of plants. Most notable is the gravity-induced downward growth of roots and the upward growth of shoots. This phenomenon, called gravitropism, has been the subject of scientific investigation since the work of Charles Darwin (Darwin, 1881). Gravitropism is of broad interest to plant scientists because it is a target trait in breeding programs that aim to improve crop nutrient and water uptake from the soil, as well as enhance light capture for photosynthesis. (Chin and Blancaflor, 2022). However, the constant presence of gravity on Earth limits the breadth of questions that can be asked about the phenomenon, particularly those that pertain to its interaction with other environmental stimuli and plant movements.



Light micrographs of hypocotyls of Arabidopsis seedlings from the F µg (A), ground controls (B = F 1g; C = G 1g), and ethylene control (D = G ethy). Note the anomalous hook that developed in hypocotyls of the light seedlings (both F µg and F 1g) and in the ethylene ground control. A typical hypocotyl hook, characteristic of dark- grown seedlings, developed in G 1g seedlings. Bar = 500 µm. (Source: NASA)

Biosatellite II experiments in the 1960s were among the first to document the effects of microgravity on plant movements. A common observation in these experiments was the occurrence of epinasty, a process in which petioles and leaves bend down (Johnson and Tibbitts, 1968). Early studies also revealed a high incidence of epinasty in plants grown on clinostats (Brown et al., 1974). Epinasty is regulated for the most part by ethylene, and levels of this gaseous plant hormone have been reported to be elevated in the Space Shuttle cabin (Guisinger and Kiss, 1999; Campbell et al., 2001) as well as in the Russian Mir Space Station. Results from the Space Shuttle and ISS have revealed microgravity-induced changes in seedling morphology and reduced expression of genes encoding ethylene biosynthetic enzymes (Kiss et al., 2000; Wakabayashi et al., 2017). These observations support the hypothesis that the epinasty that occurs during spaceflight is in part due to ethylene.

Shannon Lucid inspects wheat grown in Svet plant growth chamber on the Mir space station. (Source: NASA)

More recent studies on the ISS have led to additional insights into other plant movements that are typically dampened because of the ubiquitous nature of gravity on Earth. For example, circumnutation, a type of oscillatory plant movement, was shown to persist in weightless conditions with sunflower seedlings (*Helianthus annuus*), proving it was a true endogenous rhythm, just as Darwin suggested. But it is also dependent on gravity (Kobayashi et al., 2019). Work with coleoptile circumnutation of rice (*Oryza. Sativa*) gravitropism mutants validated earlier observations that microgravity damps circumnutation of inflorescence stems in the model plant *Arabidopsis thaliana* (Johnsson et al., 2009). By contrast, skewing and waving, phenomena observed predominantly in roots of *Arabidopsis thaliana,* or thale cress, growing on hard substrates, appear to be less influenced by gravity. This conclusion is supported by the observation that *A. thaliana* roots in the Space Shuttle and ISS still skew (Millar et al., 2011; Paul et al., 2012; Nakashima et al., 2014). Analysis of *A. thaliana* mutants in space revealed that the cytoskeleton is a component of pathways that modulate root skewing in microgravity (Nakashima et al., 2014; Califar et al., 2020).

Research on hydrotropism and phototropism has also advanced from work on the ISS. Cucumber (*Cucumis sativus*) roots displayed a stronger hydrotropic response on the ISS than on Earth. The hydrotropic response is dependent on the redistribution of the plant hormone auxin (Morohashi et al., 2017). Regarding phototropism, work with *A. thaliana* seedlings on the ISS uncovered modifications in the directional growth of roots in response to blue and red light, phenomena typically masked by gravity on Earth (Millar et al., 2010; Vandenbrink et al., 2016). Interaction between red light phototropism and fractional gravity on root growth was demonstrated by Valbuena et al. (2018). Taken together, the microgravity environment of the ISS has provided unique opportunities to expand the breadth of research on gravitropism and other tropistic responses that could benefit space crop production, as well as agriculture on Earth.

Multi-omics Approaches Provide Clues on How Plants Adapt to Spaceflight

Perhaps the most significant contribution of the ISS to plant space biology in the past decade has been the use of multi-omics approaches to better understand how plants adapt to the spaceflight environment. Omics encompasses a range of disciplines that are aimed at a global characterization of molecules to generate hypotheses about biological processes. Genomics (transcriptomics), proteomics, and metabolomics are the most used omics approaches. Among these, plant transcriptomics research on the ISS is the most popular because of rapid advances in whole genome sequencing technologies, the small amount of material needed for replicated analysis, and the establishment of a NASA-sponsored Genelab repository for gene expression datasets (https://www.nasa.gov/genelab) (Berrios et al., 2021; Meyers and Wyatt, 2022).

Most transcriptomics studies in microgravity were performed using *A. thaliana* because of the rich genomic resources that are available to study this model plant. Plant transcript profiling during the Space Shuttle and ISS era was conducted on *A. thaliana* seedlings grown in a variety of spaceflight hardware (Manzano et al., 2022). Results showed that although differentially expressed genes between space- and Earth-grown plants varied widely among studies, they fell into the broad categories of cell wall remodeling, oxidative stress, response to pathogens, and photosynthesis (Kwon et al., 2015; Johnson et al., 2017; Choi et al., 2019; Herranz et al., 2019; Kruse et al., 2020; Angelos et al., 2021). Transcriptomics using RNA-Seq also uncovered a role for alternative splicing, leading to the possibility that multiple proteins can arise from single genes during plant adaptation to spaceflight (Beisel et al., 2019; Kruse et al., 2020).

A. Thaliana plants in microgravity growing toward light. (Source: NASA)

Plant research in space has begun to consider molecular processes that might contribute to microgravity-induced differential gene expression observed in spaceflight transcriptomics experiments. DNA methylation is one process that has received attention lately because of its importance in repressing gene expression in various organisms. Indeed, methylation profiles of *A. thaliana* grown on the ISS and Chinese SJ-10 recoverable satellite were found to be different from those on Earth (Xu et al., 2018; Zhou et al., 2019). Follow-up studies revealed that spaceflight-induced epigenetic DNA-methylation modifications are heritable (Xu et al., 2021) and that the remodeling of the methylome in space overlaps with that of plant adaptation to terrestrial stresses (Paul et al., 2021).

The effects of spaceflight on the proteome of biological systems have also advanced because of the Space Shuttle program and ISS. One of the first plant proteomic studies on the ISS found that the proteins that changed in abundance in space were linked to auxin metabolism, cell trafficking, and stress responses (Mazars et al., 2014). Another study using *A. thaliana* seedlings grown onboard the ISS uncovered leaf and root proteins that differed in abundance from that of the ground controls (Ferl et al., 2015), and recent studies with *A. thaliana* seedlings found a low correlation between spaceflight-induced gene expression changes and protein levels in space that could be explained by differences in post-transcriptional modifications between spaceflight- and Earth-grown seedlings (Kruse et al., 2020). Nonetheless, like transcriptomics, proteins that changed in abundance during spaceflight were associated with oxidative stress, general stress responses, and cell wall remodeling (Ferl et al., 2015; Kruse et al., 2020).

As noted, plant spaceflight omics results on the Space Shuttle and ISS vary among different research groups, making a unified interpretation of the data challenging. Efforts that attempt to apply uniform analyses and standards should facilitate mining of plant space omics datasets for the development of crop cultivars that are better adapted to spaceflight (Barker et al., 2020; Rutter et al., 2020; Overby et al., 2021).

Cellular Responses of Plants to Spaceflight

The ISS has also enabled the elucidation of plant cellular processes that are affected by spaceflight. To this end, perhaps most significant for plant space biology are tools that enable the dynamic imaging of structures and metabolites in living plant cells using fluorescent proteins (Hilleary et al., 2018; Colin et al., 2022). Initial efforts to implement plant cell imaging on the ISS began with the development of live cell green fluorescent protein (GFP) reporters to the cytoskeleton, cytoplasmic calcium, and vacuoles, processes relevant to plant gravity responses (Dyachok et al., 2014; Toyota et al., 2018; Brillada et al., 2018). Live cytoskeletal reporters were used to document cortical microtubule orientation in seedlings of *A. thaliana* hypocotyls using fluorescence microscopy on the ISS (Soga et al., 2018) and to elucidate the role of actin filaments in autotropic root growth in simulated microgravity (DeBang et al., 2020). *A. thaliana* lines expressing genetically-encoded sensors were also used for imaging phytohormone distribution in roots on the ISS (Ferl and Paul, 2016). Furthermore, delayed vacuole fusion in hypocotyls of *A. thaliana* grown on the ISS to Earth (Wang et al., 2022). While imaging live cells on the ISS to study the effects of microgravity on plant cells remains a challenge, new hardware such as the Spectrum (see section in research facilities on the ISS below) combined with cell-specific live cell reporters (Krogman et al., 2020) offer opportunities for future plant cell biology research on the ISS.

PHYSICAL AND BIOLOGICAL CONSTRAINTS TO VIABLE CROP PRODUCTION IN SPACE

NASA's Artemis Program seeks to return humans to the Moon and to journey to Mars and beyond. As such, plant research in bioregenerative life support systems (BLSS) will be critical as humans embark on extended space missions. BLSS plant research by NASA and other international space agencies began in the 1950s, leading to recommendations of crop species and an assessment of critical environmental parameters needed for space agriculture (Wheeler, 2017; Johnson et al., 2021; Kordyum and Hasenstein, 2021). The ISS provides opportunities to evaluate crop performance and new plant growth hardware that can identify gaps and challenges for crop production in future space habitation systems (Poulet et al., 2022). This section briefly summarizes constraints for viable crop production in space through work on the ISS.

ISS Atmospheric Conditions Can Hinder Crop Performance

The atmospheric composition of the ISS differs from that of terrestrial enclosed environments, being drier (30 to 50% relative humidity) with higher CO_2 (1500 to 7000 ppm) concentrations. Additionally, ISS average temperatures are often warm (>23ºC), and the air contains many volatile organic compounds (VOCs). Chief contributors to the total trace contaminant load include methane, alcohols, and organosilicones. Other minor contributors include ketones, halocarbons, hydrogen, and carbon monoxide. Consequentially, the atmospheric composition of the ISS may have direct effects on plant growth when experiments are exposed to cabin air. As an example, during the International Shuttle-Mir Greenhouse project, wheat (*Triticum aestivum* cv. Super dwarf) was grown for an entire life cycle, resulting in approximately 300 sterile heads because of high ethylene (i.e., 0.4 ppm during anthesis) (Campbell et al., 2001). This was the first case identifying ethylene as a detrimental contaminant found in spacecraft. As noted earlier, seedling morphology and gene expression studies implicated ethylene as one factor that could affect plant growth in space environments (Kiss et al., 2000; Wakabayashi et al., 2017).

Most plant growth chambers use engineering controls (e.g., potassium permanganate sorbents like Purafil© or photocatalytic oxidation) to remove ethylene produced by plants. During rapid growth, ethylene production rates from lettuce (*Lactuca sativa*) and wheat plants range between 1.6 -2.5 nmol $m^{-2}d^{-1}$ but could be as high as 93 nmol $m^{-2}d^{-1}$ for tomatoes during fruit ripening (Wheeler et al., 2004). Plant growth chambers that circulate cabin air for cooling and the provision of CO_2 and humidity control, such as the Svet in Mir, and Lada and Veggie in the ISS, are exposed to ethylene and other VOCs from the spacecraft. Nonetheless, the current ethylene control on the ISS appears to be sufficient for good growth in open chambers like Lada and Veggie (Sychev et al., 2007). Currently, the ISS is required to maintain ethylene below 0.05 ppm. However, ethylene is seldom measured and requires specialized instrumentation to detect. Current control systems are able to scrub other trace contaminants, leading to better ISS air quality that could potentially reduce harmful effects on plants.

Elevated CO_2 can also present problems for crop growth in space. For example, high CO_2 was linked to decreased productivity in Chinese cabbage (*Brassica rapa*, cv. Tokyo Bekana) within the Veggie hardware (Burgner et al., 2020). The Advanced Plant Habitat (APH) is equipped to measure and control CO_2 and other environmental parameters, presenting a significant step for more efficient crop production in space (Massa et al., 2016; Monje et al., 2020; John et al., 2021).

Chinese cabbage plants for the Veg-03 experiment growing in Plant Pillows in the Vegetable Production System (Veggie). (Source: NASA)

Reduced Convection in Microgravity Presents Challenges for Water Provision, Nutrient Delivery, and Gas Exchange

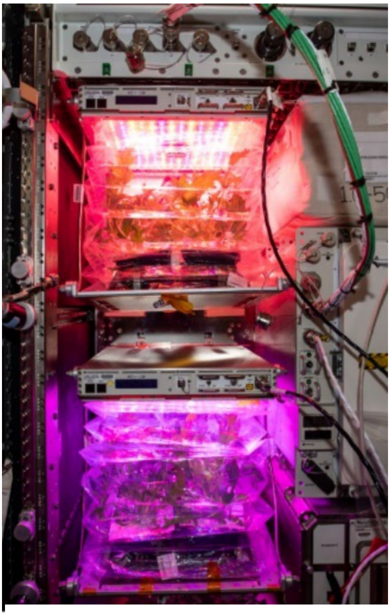
Microgravity induces physical effects that alter the microenvironment surrounding plant organs. These effects include increased boundary layers and the absence of convective mixing of atmospheric gases. In addition, altered behaviors of liquids and gases in microgravity are responsible for phase separation and for dominance of capillary forces (Porterfield, 2002). One consequence of reduced convection is root hypoxia. The lack of buoyancy-driven convection and lack of gravity-driven drainage in microgravity inhibits oxygen transport to roots by increasing the boundary layers surrounding the root zone, making oxygen supply diffusion-limited (Liao et al., 2004; Monje et al., 2004). Moisture distribution data obtained with sensors in the Svet root module clearly show that water collects at the bottom of the module in 1g. By contrast, the same amount of water is more evenly distributed throughout the root module in microgravity, potentially leading to hypoxia during spaceflight. Plants returned from microgravity were found to have experienced some degree of hypoxia stress (Stout et al., 2001), which was manifested as changes in mitochondrial ultrastructure, decreases in tissue starch reserves (Moore, 1990; Kordyum, 1994), and increases in root alcohol dehydrogenase activity (Porterfield et al., 1997; Stout et al., 2001). The occurrence of microgravity-induced hypoxia is supported by transcriptomic studies showing that some hypoxia-related genes are regulated differentially by spaceflight (Kwon et al., 2015; Choi et al., 2019).

The adverse biological effects brought about by spaceflight prompted efforts to overcome these effects in early ISS work. For example, in the Photosynthesis Experiment Systems Testing and Operations (PESTO) experiment, hypoxia was mitigated by using 1 to 2 mm arcillite media and shallow (3-centimeter-deep) root modules (Monje et al., 2005; Stutte et al., 2005). These efforts continue today with the development of passive watering systems such as the Passive Orbital Nutrient Delivery System (PONDS) (Levine et al., 2021, 2022), plant pillows (Massa et al., 2017), hydroponics, and aeroponics (Poulet et al., 2022). Testing of controlled-release fertilizers (Morsi et al., 2022) and hydrogels (soilless media with strong water and nutrient retention) (Teng et al., 2022) are also being considered as strategies to apply nutrients to crops effectively.

NASA astronauts Jessica Watkins and Bob Hines work on XROOTS, which uses liquid- and air-based techniques to grow plants rather than traditional growth media.

Light Requirements for Space Crop Production Chambers

Plants require light for photosynthesis. Therefore, information on the effects of light quality, quantity, and duration on plant development is central to plant growth habitat design. In the past, many plant growth chambers used fluorescent lamps, which provide a broad spectrum for photosynthesis and photomorphogenesis (Withrow and Withrow, 1947; Sager et al., 1982). The concept of using light-emitting diodes (LEDs) to provide lighting for the growth of plants in space (Ignatius et al., 1991; Bula et al., 1991; Barta et al., 1992; Morrow et al., 1994) was a major advancement. LEDs do not radiate much heat, are easily dimmable and have a long operating life. As such, LEDs have appeal for space crop production applications. Moreover, they do not contain mercury vapor found in fluorescent lamps, which can be a safety concern in space.



Two Vegetable Production Systems (Veggies) use red-rich and blue-rich light treatments to grow Mizuna mustard during the Veg-04B experiment. (Source: NASA)

A challenge with LEDs is that the spectral output is very narrow (approximately 25 nm half band width), so determining ways to provide adequate spectral combinations became a major focus of research. Studies demonstrated that both red and blue light are essential for normal growth and development for many plant species (Hoenecke et al., 1992; Kim et al., 2007). Since their early use for growing plants in the 1990s, LED electrical efficiencies have improved greatly (Bourget, 2008), and recent studies on space crop candidates indicate that response to LEDs is species-specific, potentially guiding the future development of lighting systems for plant growth chambers in space (Mickens et al., 2018, 2019; Graham et al., 2019).

Regardless of the source, the requirement for light to sustain plant growth makes plant chambers and plant testing in space more challenging for thermal management. Even though some of the light is fixed by the plants into biochemical energy through photosynthesis, from a thermal management perspective, nearly all the power required to generate lighting in a plant chamber ultimately becomes heat. Hence, plant chambers and experiments for space require adequate air mixing and sufficient cooling capacity to maintain temperature control. These requirements often translate into the need for more electric power in plant growth chambers in space for than those on Earth. However, many of the new LEDs have an electrical conversion efficiency of 30-45%, leading to a lower fraction of electricity going directly to heat. Furthermore, heat is removed through air or fluid cooling loops on ISS, reducing the risk of high temperature impacts on plant growth.

Most plant experiments to date have been conducted at low to moderate light levels (75-300 µmol m^{-2} d^{-1} of photosynthetically active radiation; PAR) because of constrained power allotments to spaceflight plant growth chambers. Stutte et al. (2005) found no difference in the rates of photosynthetic carbon uptake, water loss via transpiration growth between flight and ground plants at moderate light levels (~300 µmol m^{-2} d^{-1}), and saturating CO_2 concentration. These results suggest that food crop production rates do not change in space because the underlying biological processes operate at the same rates as in 1g at those light levels (Monje et al., 2005). However, this finding does not mean that direct and indirect effects of microgravity cannot affect plant growth at higher, more demanding light levels (e.g., 600 to 900 µmol m^{-2} d^{-1}).

In previous studies, a significant (approximately 17 percent) reduction in whole-chain electron transport was observed in chloroplast extracts from plants raised in microgravity at high light levels (Tripathy et al., 1996). These higher light levels translate into faster plant growth rates, which result in proportionally higher rates of water demand and greater rates of respiratory O_2 consumption by both shoots and roots. In turn, these higher rates impose greater loads (e.g., greater water supplied to the root zone, greater dehumidification capacity, and increased oxygen consumed in respiration) that could cause plant stress if the flight hardware cannot control or sustain mass exchange rates. The APH has been designed to accommodate plant experiments at higher light intensities (Massa et al., 2016). Its development demanded improved technologies to provide higher capacity water supply and condensate recovery, heat rejection, and CO_2 control.

Hatch green chile plants are pictured growing in the Advanced Plant Habitat (Plant Habitat) aboard the International Space Station. (Source: NASA)

Plant Microbes: Telling Friend from Foe in Future Space Crop Production Systems

NASA's interest in microbiology increased rapidly in the past few years not only because of implications for crew health, food safety, and plant disease control (Lee et al., 2021; Schueger et al., 2022), but also for the potential of using the microbiome to enhance crop resilience under stressful environments (Trivedi et al., 2022). Enclosed environments in space are conducive for harmful pathogens to infect plants, potentially threatening crop productivity in BLSS applications (Schueger, 1998; Schueger et al., 2022). Studies of wheat grown on the Space Shuttle showed seedborne fungal infection of leaves by *Neotyphdium* sp. (Bishop et al., 1997). Soybean seedlings on another Space Shuttle mission were also found to be more susceptible to *Phytophthora sojae* than those on Earth (Ryba-White et al., 2001). More recently, *Zinnia hybrida* (cv. Profusion) grown on the ISS developed necrotic symptoms caused by the opportunistic fungus *Fusarium oxysporum* (Schueger et al., 2021).

To mitigate future plant pathogen epidemics in BLSS applications, methods of monitoring microbes by meta-transcriptomics, remote assessment of plant health, and seed sanitation are being explored by NASA (Poulet et al., 2022; Dixit et al., 2021). The pocket-sized nanopore MinION platform is one technology that has potential applications in this regard. MinION was used to characterize the microbial communities in leaves of red romaine lettuce under ISS-like conditions (Haveman et al., 2021) and as a diagnostic tool for pathogenic fungi in *Zinnia* plants grown on the ISS (Haveman et al., 2022). Metagenomics has already been used to demonstrate the absence of food-borne human pathogens in ISS-grown lettuce and mixed crops (Khodadad et al., 2020; Hummerck et al., 2021). Furthermore, microbial sequencing technologies on the ISS identified members of the *Sphingomonas* genus, which are plant growth promoting bacteria that could be harnessed for space crop production (Lombardino et al., 2022). Plant microbial research on the ISS is still in its infancy. However, technological advances in metagenomics promises to accelerate research in this area.

The Veg-01 investigation assessed on-orbit function and performance of the Veggie facility, focusing on the growth and development of Zinnia plants in the spaceflight environment and the composition of microbial flora on the plants and the facility. (Source: NASA)

Research Facilities and Equipment on ISS and How to Choose Them

Design Considerations for Plant Growth Systems to be used in Space Exploration

1. The design of biological experiments (e.g., cells, plants, animals, etc.) conducted in microgravity must account for: 1) changes in gravity-dependent fluid and gas behavior, 2) potential effects of spacecraft atmosphere, and 3) hardware-specific limitations: ventilation, light level, CO_2 supply, humidity and temperature control, and ethylene removal.
2. In the absence of convective gas mixing, the secondary effects associated with increased resistance across the boundary layer for gas exchange, reduced capacity of evaporative heating, and accumulation of volatiles in localized areas require that careful attention be paid to the design solution and provision of adequate air mixing.
3. Leaf gas exchange is not affected directly by microgravity, but indirectly by the lack of buoyancy-driven convective currents that limit diffusion of gases to the leaf. As such, it is important to ensure that sufficient rate of air mixing is occurring in the plant chamber.
4. Water distribution within rooting media is more homogenous because capillary forces dominate (i.e., there is no gravitational driven drainage), which can cause poor aeration.
5. Without any forced air circulation, heat and mass transfer are sustained only by diffusion.

Widely-used plant growth facilities and equipment are discussed in the following pages.

Additional information on plant facilities and equipment can be found at www.nasa.gov/stationfacilities.

Plant Growth Facilities

Biological Research in Canisters (BRIC)

ESA Astronaut Samantha Cristoforetti activates all four BRIC-21 experiment canisters by injecting the growth medium to the samples. (Source: NASA)

BRIC canisters provide a carrier to house Petri plates of various sizes to accommodate study of tissue or cell cultures, microbes or other organisms that could be contained in a dark environment. A series of BRIC hardware exists to accommodate various experiment requirements. These include the BRIC-60, which has two compartments that each have the capacity for 12 60-mm Petri dishes; BRIC-100, which is a single anodized-aluminum cylinder that can accommodate up to nine 100 mm Petri plates; and the BRIC-100 (VC), which has additional structural support for vacuum containment of specimens.

BRIC Petri Dish Fixation Unit (BRIC/PDFU) and BRIC-LED

A more sophisticated version of studies using Petri dishes can be carried out with the BRIC/PDFU hardware, which can also accommodate up to 2 fluids, growth media to initiate experiment, and chemical fixatives for *in situ* fixation of the tissue. The PDFU is a specialized holder for a 60-mm Petri dish and reservoir for the containment and delivery of growth media and fixative. Each BRIC canister can contain up to six individual PDFUs. The BRIC-LED system was designed to complement the capabilities of the existing BRIC-PDFUs by the addition of customizable discrete lighting that illuminates the individual 60 mm petri dishes. Four different wavelengths of LEDs are available for each petri dish (blue, red, far-red and white) and are configured as specified by the investigator.

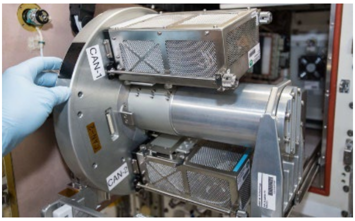
Kennedy Fixation Tube (KFT)

Kennedy Fixation Tube (KFT) (Source: NASA)

Experiments performed on ISS frequently require the experimental organisms to be preserved until they can be returned to Earth for analysis. The KFT was developed to allow astronauts to apply fixatives – chemical compounds that are often toxic – to biological samples without the use of a glovebox while maintaining three levels of containment. KFTs have been used over 200 times on orbit with no leaks of chemical fixative. The KFT is composed of a polycarbonate main tube where the fixative is loaded preflight, the sample tube where the plant or other biological specimen is placed during operations, plus an expansion plug, actuator, and base plug that together provide fixative containment. The main tube is pre-filled with 25 mL of fixative solution pre-flight. When actuated, the specimen contained within the sample tube is immersed with approximately 22 mL of the fixative solution. The KFT was demonstrated to maintain its containment at ambient temperatures, 4°C refrigeration and -100°C freezing conditions.

Astronaut Mark Vande Hei photographs and harvests Plant Habitat-05 Experiment Plates during Expedition 66. (Source: NASA)

Plant Experiment Unit/Cell Biology Experiment Facility (PEU/CBEF)



A Plant Experiment Unit (PEU) is inserted into the Cell Biology Experiment Facility (CBEF) during Plant Rotation sample preparation. (Source: NASA)

The PEU is used within the CBEF for plant life-science experiments on ISS. CBEF is used for a variety of life science experiments. It consists of an incubator unit and a control unit. The incubator unit consists of a μ *g* compartment and a 1 *g* compartment. The 1 *g* compartment has a centrifuge that can generate artificial gravity from 0.1 to 2.0 *g*. Experiment units (such as the PEU – Plant Experiment Unit) are set in containment canisters and installed in the CBEF. The CBEF can control temperature (15-40°C), humidity (20-80%) and CO_2 concentration. The PEU enables plant growth experiments from seed to seed and includes the following components: (1) a plant growth container, (2) a water delivery control system, (3) an air ventilation control system, (4) an illumination system (LED), (5) a CCD camera, (6) a temperature sensor and (7) a humidity sensor. Researchers can monitor operational data such as temperature, humidity and water content of the plant bed, and observe real-time images of the growing plant. Experiment parameters can be set to run automatically and can be changed remotely from the ground.

Vegetable Production System (Veggie)

NASA astronaut Shane Kimbrough during the first harvest of Outredgeous Red Romaine Lettuce from the Veggie facility during VEG-03. This first of four harvests is part of a new paradigm of harvesting called "Cut and Come Again" where the astronaut harvests the outer leaves, allowing for longer growth of the plants. (Source: NASA)

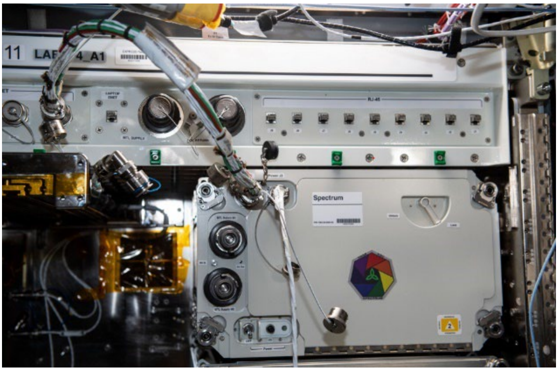
Veggie is a simple expandable plant growth unit with an LED (red 630 nm, green 530 nm, blue 455 nm) lighting system that provides up to 500 µmol $m^{-2}s^{-1}$ of photosynthetic active radiation (PAR). In addition to growing vegetables in space, Veggie was used to support Petri Plate science experiments that included monocot and dicot plant species that were grown for pre-determined durations, photographed, harvested, and preserved for return to Earth. Air temperature, humidity, and CO_2 provision for photosynthesis are achieved by drawing ambient cabin air in through the system. The total plant growing area is 0.13 m^2 and the maximum height for growing plants is 47 cm. The baselined procedure for growing plants in Veggie uses root pillows containing substrate, slow-release nutrient pellets and seeds. Water is administered on orbit to initiate seed germination and is periodically added throughout the growth cycle. Other watering and nutrient delivery capabilities for the Veggie platform continue to be developed. Examples are PONDS and Advanced Plant Experiment (APEX) Growth Chamber, which provide plant growth hardware that can house substrate, nutrients, and seeds. Water is administered on orbit to initiate seed germination and is periodically added throughout the growth cycle.

Amara Mustard plants are pictured growing inside the Veggie space botany research facility. (Source: NASA)

Spectrum (Multispectral Fluorescent Imager)

Spectrum is a multispectral fluorescence imager designed for capturing *in vivo* gene and protein expression for a variety of biological organisms on ISS (e.g., organisms transformed with genetically-encoded *in vivo* reporters based on fluorescent proteins). Spectrum accommodates removable 10 cm x 10 cm Petri plates, various sized multi-well culture plates, and other custom containers within the growth chamber. Spectrum provides programmable temperature (18°C-37°C), CO_2 control (400 ppm up to ISS-ambient levels), photoperiod regulation, image capture, ethylene scrubbing (< 25 ppb), and directed air flow to prevent condensation that would interfere with imaging. Spectrum also provides automated chamber management and continuous collection of chamber environment information while capturing 71 megapixel images with resolution to 2.5 µm. A 10-position motorized filter wheel contains fluorescence emission filters paired with an Excitation Light Source (ELS) that uses diffusers to achieve 80% uniform illumination of the target area.

Spectrum allows on-orbit change-out of filters. The growth light cap is equipped with broad-spectrum white (400-750 nm), red (630-660 nm), blue (400-500 nm), and green (520-530 nm) LEDs that illuminate samples from the top with an intensity range of 0-1000 $\mu mol\ m^{-2}s^{-1}$. Spectrum can be commanded from the ground and stores time stamped images with the option to downlink data for near-real-time evaluation. The operational software can be modified during flight.

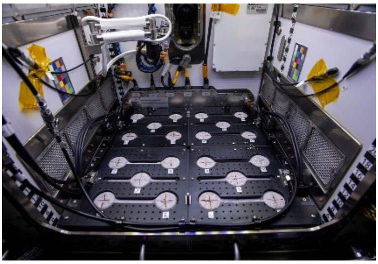


Spectrum takes fluorescent images of biological specimens contained within a controlled environment. A high resolution, monochrome camera captures images of fluoresced proteins of plants within Petri plates. (Source: NASA)

Advanced Plant Habitat

APH is a large growth volume habitat designed for multi-generational and developmental plant studies on ISS. The habitat is equipped with more than 180 calibrated sensors to ensure the system functions autonomously using a pre-programmed experiment design that provides a suitable growth environment (e.g., temperature [18-30°C], relative humidity [50-90%], CO_2 concentration [400-5,000 ppm], ethylene scrubbing [<25 ppb], air ventilation flow [0.3-1.5 m/s] and controlled light intensity and spectral quality) for plant experiments up to 135 days.

The APH growth chamber supports plant growth up to 45cm (maximum shoot height), with a growth area consisting of 1,708 cm^2 and a growth volume of 112,500 cm^3. A removable science carrier tray contains four independently-controllable root modules with each module containing two moisture sensors and one subsurface O_2 sensor with water/nutrient delivery (up to 2 L/day) provided through four porous tubes in each module. The science carrier supports a root zone height up to 5 cm and can be configured with substrates and nutrients as specified by the researcher. Air and water samples can be collected during experimentation and returned to the researcher for evaluation.



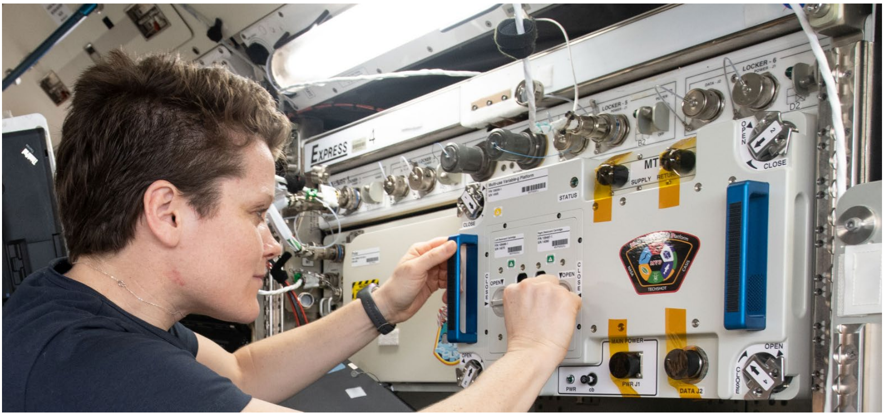
View of the Advanced Plant Habitat (APH) at the start of Plant Habitat-02. (Source: NASA)

APH contains two color cameras (top and side views) and one infrared camera (for dark cycle imaging) that can capture periodic and time-lapsed still images for monitoring plant growth. Growth lighting is designed with a controllable photosynthetic photon flux (PPF) of 0-1000 µmol $m^{-2}s^{-1}$ (photosynthetically active radiation 400-750 nm) with an LED light assembly consisting of broad spectrum white (0-600 µmol $m^{-2}s^{-1}$ ±5%, 400-700 nm), red (0-600 µmol $m^{-2}s^{-1}$ ±5%, 630-660 ±10 nm), blue (0-400 µmol $m^{-2}s^{-1}$ ±5%, 400-500 ±10 nm), green (0-100 µmol $m^{-2}s^{-1}$ ±5%, 525 ±10 nm), and far-red (0-50 µmol $m^{-2}s^{-1}$ ±5%, 730-750 ±10 nm) lighting uniformity as measured 15 cm below the growth light assembly and 5 cm away from a wall.

Radishes flourish in the APH during the Plant Habitat-02 experiment. (Source: NASA)

Multiuse Variable-G Platform (MVP)

MVP contains dual 390 mm rotors that can provide two independent gravity level treatments (0-2 *g*). It provides thermal control and ground telemetry in real time for data review. There are 12 sample modules (6 per centrifuge) with video capability and custom modules to support plants, bacteria, cell cultures, *Drosophila, C. elegans*, etc. Rotors and sample modules are removable on orbit. Each plant module supports three Petri plates and provides LED lighting, a CO_2 sensor, circulation and blower fans, and a camera for plant imaging.



NASA astronaut Anne McClain installs the Multi-use Variable-g Platform 2 (MVP-2) platform onto Express Rack 4. (Source: NASA)

Cube Payloads for ISS Experiments

Facilities operated by Space Tango and Nanoracks on ISS support Cube payloads. Both facilities are fully automated and allow multiple payloads to run simultaneously and independently on orbit. They provide a standardized platform and open architecture for Cube payload modules of different configurations that can be managed from the ground with downlink of images and data in near real-time. Subsystems include fluid control, temperature control, imaging and observation. Customizable variables for plant studies include growth light intensity, growth light cycle, growth medium recipe, imaging intervals and timing. Space Tango has a Powered Ascent Utility Locker (PAUL) to accommodate Cube experiments that need continuous power during launch to orbit. Accommodations are available for 1U, 2U,4U, 6U, and 9U payloads.

Example Nanoracks Cube payload module. (Source: NASA)

XROOTS (eXposed Root On-Orbit Test System) - Under Development

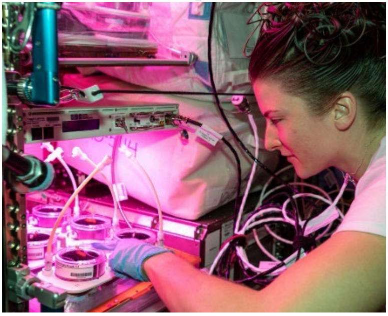
The eXposed Root On-Orbit Test System (XROOTS) grows plants in the microgravity environment of the ISS and evaluates nutrient delivery and recovery techniques such as aeroponics over the course of a full plant growth cycle. (Source. NASA)

XROOTS uses hydroponic and aeroponic techniques to grow plants without soil or other growth media. There are multiple independent growth chambers that are imaged from seed germination onward.

The existing baseplate in the Veggie unit is replaced with the XROOTS enclosure, and a custom bellows replaces the standard Veggie bellows. XROOTS uses power and data feeds from the EXPRESS rack as well as thermal conditioning. XROOTS is designed to test variables such as nutrient delivery and usage, nutrient recovery, power utilization, spray characteristics and protocols, and root zone atmosphere. Once validated under conditions of microgravity, XROOTS would be available for crop production for future space missions.

Passive Orbital Nutrient Delivery System (PONDS) - Under Development)

PONDS is a new plant growth approach that contains an area for a contained plant growth substrate and a reservoir for water and/or plant nutrient solutions. It was developed to fit under the Veggie light bank. PONDS provides water delivery to seeds for germination and fulfills the requirement to transport water from the reservoir for plant growth while providing aeration to the root zone under both 1g and μg conditions. The PONDS on-orbit concept of operations is similar to Veggie plant pillows. A clear germination cap maintains high humidity levels during the seed germination phase. The plant cylinder is removed at harvest, placed in a bag for cold stowage and returned. The PONDS reservoir has a separate opaque cap that is installed prior to return. Once protocols are validated, procedures can be developed so that PONDS units can be reused for successive grow-outs.



NASA astronaut and Expedition 66 Flight Engineer Kayla Barron checks out plants growing inside the Veggie botany research facility for the Veggie PONDS experiment. (Source: NASA)

Support Facilities on ISS

The ISS has a variety of multidisciplinary laboratory facilities and equipment available to support science operations. These capabilities were built by NASA and its International Partners and can be made available on a time-shared basis to other U.S. government agencies and private entities to pursue their own mission-driven research and applications.

Temperature-controlled stowage of samples is available in the Minus Eighty-Degree Laboratory Freezer for the ISS (MELFI) and the General Laboratory Active Cryogenic ISS Experiment Refrigerant facilities. Fixation of tissues for microscopic and/or molecular analysis is an option. There is a suite of facilities and instrumentation currently available to support plant experiments on the ISS including the Microgravity Science Glovebox for handling hazardous materials in space. In addition to NASA provided hardware, facilities from experiment implementation partners are also available such as the Nano-racks Microscopes and Nano-racks Plate Reader.

Astronaut Nick Hague is photograph as he transfers samples from one Glacier to another Glacier using Ice Bricks and a Coldbag. (Source: NASA)

Descriptions of these facilities and their capabilities can be found at a number of different websites including the NASA ISS facilities website at www.nasa.gov/stationfacilities/ and NASA's Fundamental Space Biology Science Plan at https://www.nasa.gov/pdf/541222main_10-05-17%20FSB%20Sci%20Plan-Signed_508.pdf

Funding, Developing, and Launching Research to ISS

Finding a Sponsor

Every experiment on the space station needs to be sponsored and funded to be developed, integrated, flown, and operated onboard. Several sources of funding are available to scientists for research, payload development, payload processing at NASA facilities, on-orbit operation, and more.

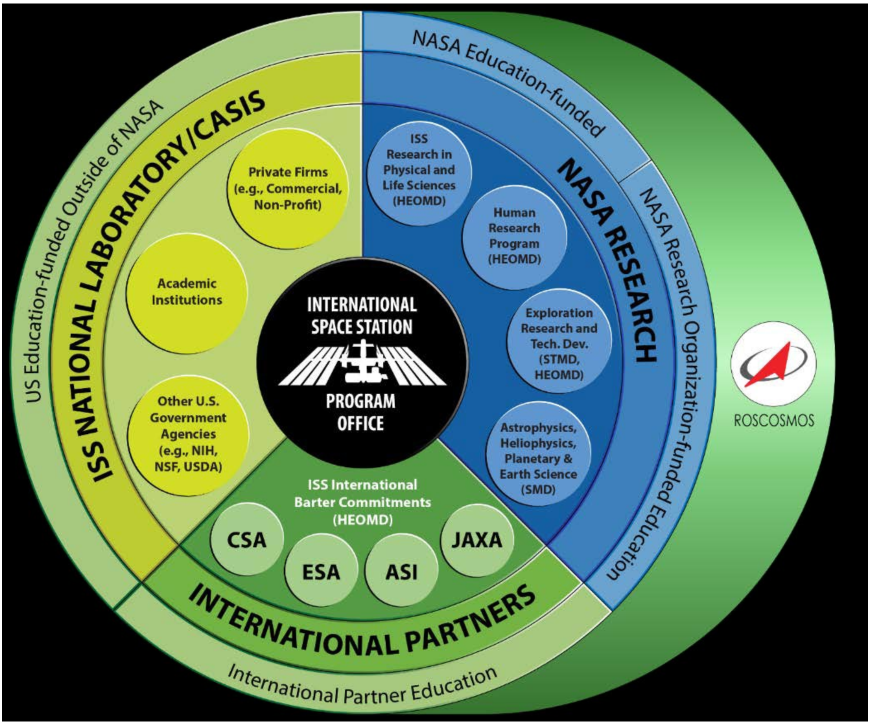
In general, NASA funding for space station use is obtained through NASA Research Announcements (NRAs). Funding from other government agencies, private, and non-profit entities to use the space station is obtained through research opportunities released by ISS U.S. National Laboratory, otherwise known as the Center for the Advancement of Science in Space (CASIS). Space Station International Partner funding can be obtained through their respective agencies.

ISS U.S. National Laboratory

In 2011, NASA finalized a cooperative agreement with CASIS to manage the International Space Station U.S. National Laboratory (ISS National Lab). The independent, nonprofit research management organization ensures the station's unique capabilities are available to the broadest possible cross section of U.S. scientific, technological and industrial communities.

The ISS National Lab develops and manages a varied research and development portfolio based on U.S. national needs for basic and applied research. It establishes a marketplace to facilitate matching research pathways with qualified funding sources and stimulates interest in using the national lab for research and technology demonstrations and as a platform for science, technology, engineering and mathematics education. The goal is to support, promote and accelerate innovations and new discoveries in science, engineering and technology that will improve life on Earth.

More information on ISS National Lab, including proposal announcements, is available at www.issnationallab.org.



Other Government Agencies

Potential funding for research on the ISS is also available via governmental partnerships with ISS U.S. National Laboratory and includes (but is not limited to) such government agencies as:

- Department of Energy (DOE)
- Department of Defense (DOD)
- National Science Foundation (NSF)
- National Institutes of Health (NIH)
- U.S. Department of Agriculture (USDA)
- International Funding Sources

Integral to the ISS are the partnerships established between the United States, Russia, Japan, Canada and Europe. All partners share in the greatest international project of all time, providing various research and experiment opportunities for all. These organizations – JAXA (Japan Aerospace Exploration Agency), Canadian Space Agency (CSA), ESA (European Space Agency), Russian space agency Roscosmos, Centre National d'Etudes Spatiales (CNES), and the German Aerospace Center (DLR) – provide potential funding opportunities for international scientists from many diverse disciplines.

ISS Commercial Opportunities

An additional method to conduct research and other activity on the ISS is through commercial activity. NASA has opened the International Space Station (ISS) for business to enable commercial and marketing opportunities on the microgravity laboratory. Since then, there has been a growing demand for commercial and marketing activities from both traditional aerospace companies and from novel industries, demonstrating the benefits of the space station to help catalyze and expand space exploration markets and the low-Earth orbit economy.

NASA's Commercial LEO Development Program supports the development of commercially owned and operated LEO destinations from which NASA, along with other customers, can purchase services and stimulate the growth of commercial activities in LEO. As commercial LEO destinations (CLDs) become available, NASA intends to implement an orderly transition from current International Space Station (ISS) operations to these new CLDs. Transition of LEO operations to the private sector will yield efficiencies in the long term, enabling NASA to shift resources towards other objectives. With the introduction of CLDs, NASA expects to realize efficiencies from the use of smaller, more modern and efficient platforms and a more commercial approach to meeting the Agency's needs in LEO. In the longer term, the gradual emergence of additional customers for commercial LEO destinations will offer the opportunity for additional savings.

The extension of ISS operations to 2030 will continue to return benefits to the United States and to humanity as a whole while preparing for a successful transition of capabilities to one or more commercially owned and operated LEO destinations (CLDs). NASA has entered into a contract for commercial modules to be attached to a space station docking port and awarded space act agreements for design of three free-flying commercial space stations. U.S. industry is developing these commercial destinations to begin operations in the late 2020s for both government and private-sector customers, concurrent with space station operations, to ensure these new capabilities can meet the needs of the United States and its partners.

Working with NASA

Once a payload has been selected for development, engineering and operations staff in the ISS Program Office are available to work with payload teams through the design, test, certification, build, and launch phases prior to beginning mission operations on ISS. More detailed information on this process, and information on current and planned launch vehicles, is available at https://www.nasa.gov/stationopportunities.

Potential proposers to any NASA program announcement should contact the relevant Program Scientist to discuss the appropriateness of their concepts for the specific solicitation and to determine who to contact within the ISS Program Office for discussing expected development costs for their proposal budgets.

Hatch chili peppers were grown during the PH-04 experiment, which was conducted for 137 days in 2021. It was the longest - and spiciest - plant experiment to date on the orbital laboratory. (Source: NASA)

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Acronyms

APH	Advanced Plant Habitat
APEX	Advanced Plant EXperiment
BLSS	Bioregenerative Life Support Systems
BRIC	Biological Research in a Canister
CASIS	Center for the Advancement of Science in Space
CBEF	Cell Biology Experiment Facility
CGBA	Commercial Generic Bioprocessing Apparatus
EC	Experiment Container
ISS	International Space Station
LED	Light-Emitting Diode
MVP	Multiuse Variable-G Platform
NL	National Lab
NSPIRES	NASA Solicitation and Proposal Integrated Review and Evaluation System
PDFU	Petri Dish Fixation Unit
PESTO	Photosynthesis Experiment and System Testing and Operation
PEU	Plant Experiment Unit
PH	Plant Habitat
PI	Principal Investigator
PONDS	Passive Orbital Nutrient Delivery System
RH	Relative Humidity
USU-Apogee	a type of dwarf red wheat
Veggie	Vegetable Production System
VOC	Volatile Organic Compounds
XROOTS	eXposed Root On-Orbit Test System

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