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Plants in Space

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Abstract

Plants will play a critical role in the survival of human beings on long-duration space missions, probably beginning pretty soon with a mission to Mars. Plants can adapt to extreme environments on Earth, and model plants have been shown to grow and develop through a full life cycle in microgravity. In space, long-term human space exploration missions require a life support system in which higher plants play a vital role. Growing crops in space is as much about developing the humans' technological capacity to provide plants with adequate growth conditions in the unique microgravity environment, as is about the symbiotic relationship between plants and space travelers. After several decades of research, we have learned a lot about the impediments to growing plants in microgravity, in outer space, and on other planets. As human space exploration advances, we should feel confident about our ability to grow plants on board spacecraft during long-term space missions, on the Moon, and on other planets. Plants will require specialized environments for growth and development in microgravity, but – at least on a small scale – we already know how to produce such growth chambers and greenhouses.

Keywords: space biology, gravitational biology, microgravity, plants, spaceflight, international Space Station

1. Introduction

The phrase “plants in space” refers to plants that are grown in the physical universe known as outer space, a region beyond the Kármán line in the Earth's atmosphere, at an altitude of approximately 200–450 km above sea level, which is the typical orbit range of the Space Shuttle missions and of the International Space Station, where most of human spaceflight and research has taken place [1]. Outer space represents a challenging environment for human exploration for a number of reasons, including the lethal hazards of extreme temperatures, high vacuum, electromagnetic radiation, particle radiation, and magnetism. A deep understanding of the

biological consequences of exposure to the space environment is required to design efficient countermeasures to minimize their negative impact on living organisms, humans and plants alike. In addition, the economic cost of sending anything into space is very high. In outer space, plants are typically grown in a microgravity (often referred to as weightlessness) controlled environment, in specific space plant growth chambers.

Plant space biology has been closely associated with human space exploration in that plants are considered as key parts of biologically based life support. Learning to grow plants in space is an essential goal for long duration space missions since crop growth in space will be beneficial in a variety of ways, aiding with air regeneration, food production, and water recycling [2–6]. The logistical challenges of the long-term human space exploration missions require a self-sustainable life support system. Traveling in a spacecraft to other worlds will put constraints on the quantity and weight of commodities that could be brought along. In that context, higher plants are of paramount importance for providing *in situ* resource utilization through a continuous supply of fresh food, atmosphere revitalization, and clean water for humans.

The many challenges of spaceflight research have logistical and resource constraints, including significant limitations on available space, power, crew time, cold stowage, and data downlinks. Additional issues are related to hardware development, safety concerns, and the engineering versus science culture in space agencies. There is not much space for growing plants in space. Concerning research, the difficulties of publishing the results from spaceflight research stem from the lack of adequate controls, limited sample size, the frequent impossibility for verification of the obtained data, and the indirect effects of the spaceflight environment.

The concept of growing crops in space is as much about developing the humans' technological capacity to provide plants with adequate growth conditions in the unique microgravity environment, as it is about the symbiotic relationship between plants and space travelers. Plants in space provide numerous benefits to the humans that accompany them. They improve the quality of indoor air by helping control humidity levels, and by removing and converting the carbon dioxide from air into essential oxygen that humans can breathe. Central to the concept of regenerative life support systems for space exploration is the use of photosynthetic organisms and light to generate oxygen and food. It is also axiomatic that plants can be consumed as food, providing a nutritive value to organisms throughout the food chain. Growing plants in space may also provide psychological and neurocognitive benefits to the human spaceflight crews, in the form of therapeutic people-plant interactions [7].

2. Fundamentals

Humans began the physical exploration of space during the 20th century with the advent of high-altitude balloon flights, followed by rocket launches. Plants have been used in space experiments since the early days of the space program. The early suborbital launches saw the first organisms in space, as unspecified “specially developed strains of seeds”, sent to 134 km altitude above Earth on July 9, 1946, on a U.S. launched V-2 rocket; these samples were not

recovered. The first seeds launched into space and successfully recovered were “ordinary corn seeds”, launched on July 30, 1946; see [8] for a descriptive chronology of the early biological experiments in rockets. These early and very brief biological experiments were primarily concerned with the effects of radiation exposure on living tissue, including seeds. Some years later, the first plant materials taken into a microgravity environment for a longer ride in orbit were seeds of wheat, pea, maize, and onion, flown on board of Sputnik 4 in 1960 [2]. The first full life cycle of a plant (*Arabidopsis thaliana*) in space was completed on Salyut-7, resulting in clearly observable developmental alterations and in some viable seed, but mostly in seed having nonviable embryos [9].

Opportunities for space experiments greatly increased by the initiation of scientific operations in orbital laboratories, the latest being the International Space Station (ISS). Building upon accumulated knowledge, researchers took advantage of well-developed plant growth chambers for microgravity, which in general provided a very good environment for growing plants on the ISS [10]. These proved that it is possible to have plants pass the full cycle of ontogenesis in space (on the ISS), and to produce plants and viable seed similar to the ground controls. The first example of seed-to-seed-to-seed (i.e., two consecutive life cycles) of a plant (*Arabidopsis thaliana*) in space was completed in 2000–2001 [11]. With advanced plant growth chambers that in general provided a well-regulated environment for growing plants in microgravity on the ISS, most of the problems seen in previous plant spaceflight experiments were successfully eliminated. It turns out that gravity is not necessary for seed-to-seed growth of plants, though it plays a direct role in plant form, and may influence seed reserves [11].

The effort and resources allocated to plant cultivation in space have revealed many answers, while at the same time raising new research questions. Periodic literature updates on the status of plant space biology have reviewed the documented influence of gravity on both plant growth and development, and specifically on a myriad of cellular and molecular responses, including cell cycle, embryogenesis and seed development, photosynthesis and gas exchange, gravitropic sensing and response, phototropism, cell wall development, and gene expression changes [12–15]. More recent and also more sophisticated plant experiments during the Space Shuttle and the ISS era produced key science insights on the molecular and cellular mechanisms underlying biological adaptation to spaceflight, and especially to plant growth, development, tropisms, and stress responses in microgravity [16–19].

3. Particulars

The first experiments with higher plants grown in space were intended to assess whether plants could grow outside Earth and to determine what differences there were between spaceflight-grown and Earth-grown plants. As plant-growth hardware started to adapt to spaceflight, opportunities were created for more sophisticated plant experiments. Direct microgravity effects started being differentiated from confinement effects, and Earth orbit became a laboratory where plants could be grown without the influence of Earth gravity.

The physiological effects of gravity range from subtle to substantial, and influence numerous molecular and cellular events in addition to those solely associated with gravitropism. Many of the early plant space biology experiments resulted in morphological and physiological changes, manifested as cellular and phenotypic abnormalities. These include chromosomal breakage [20], failure to produce seed [21], altered or nonviable embryos [9], alterations in cell wall composition and properties [22], increased breakdown of xyloglucans [23], changes in polar auxin transport [24], or other morphological abnormalities [25]. Indeed, spaceflight appears to initiate both molecular and cellular remodeling throughout the plant. For example, spaceflight can induce significant genomic and epigenomic mutations [26]. In the absence of gravity plants rely on other environmental cues to initiate the morphological responses essential to successful growth and development, and the basis for that engagement lies in the differential expression of genes in an organ-specific manner [11, 16, 27], which is followed by a microgravity-driven remodeling of the proteome [28].

Reflecting on the early spaceflight experiments, we now know that a number of the early obtained results were more likely due to the rigors of the microgravity environment than to the lack of gravity itself. For example, altered starch content has been reported for different species of space grown plants: pepper [29], lepidium [30], maize [31], and *Arabidopsis* [32]. However, just improving plant ventilation during space flight was found to eliminate carbohydrate differences [33]. In addition, ethylene, a plant stress hormone, is a common problem in microgravity experiments. Plant ethylene production increases in space [34]. Elevated ethylene levels (1100–1600 ppb on a Shuttle) caused anomalous seedling growth of *Arabidopsis* in spaceflight studies, although they had no effect on relative graviresponsiveness [35]. Furthermore, ethylene levels on the MIR space station were very high (800–1200 ppb) during a Brassica spaceflight growth study [36]. While Brassica plants were capable of producing seed at this ethylene level, the same environment stopped a wheat crop from producing seed on board MIR [37]. Novel plant growth spaceflight hardware uses ethylene scrubbers to mitigate the negative effects of elevated ethylene levels in spacecraft [10, 11].

The absence of natural convection in space makes it easy for plants to become oxygen starved [38]. Hypoxia symptoms in seed include reduction in size of the protein bodies, failure of the protein bodies to fill, free floating lipid droplets in the cytoplasm, abnormally vacuolated cells, and degeneration of portions of the embryo. In a full life-cycle spaceflight experiment with Brassica, the protein bodies that were found to be 44% smaller, starch grains were aberrantly deposited in the seed, and the cotyledon cell number was reduced by 80% [36]. This study concluded that alterations in the oxygen and ethylene concentrations within developing siliques were problematic in the experiment [36]. While the Svet greenhouses used to grow Brassica on MIR used a fan to circulate air, the circulation rate was insufficient (below 0.5 m/s) to prevent hypoxia [38]. Control of the gaseous environment appears to be a key factor for plant reproduction in microgravity [39].

3.1. Plants for bio-regenerative life support systems

The logistical challenges of long-term human space exploration missions require a life support system capable of regenerating all the essentials for survival. The life support systems on the

ISS provide oxygen via water electrolysis, absorb and remove carbon dioxide, and manage vaporous emissions (e.g., ammonia, acetone) from the astronauts themselves; water is recycled.

Central to the concept of bio-regenerative life support systems is the use of photosynthetic organisms and light to generate oxygen and food. Learning to grow plants in space is thus an essential goal for long duration space missions since crop growth in space will aid with air regeneration, food production and water recycling for astronauts during long-term space missions [2, 40]. Research on plants in space, in addition to producing key scientific insights into specific plant gravitropic and abiotic stress responses, fosters the overall development of bio-regenerative life support systems for the production of oxygen, food, and nutrients [41].

The cultivation of higher plants occupies an essential role within bioregenerative life support systems (BLSS), which are designed to provide a habitation environment similar to the Earth's biosphere for space missions with extended durations and in deep space. It contributes to all key functional aspects by closing the different loops in a habitat like oxygen production, carbon dioxide reduction, food production, water management, and metabolic waste recycling. Fresh crops are also expected to have a positive impact on crew psychological health.

Different designs and technological solutions have been implemented in higher plant flight experiments. Continuous subsystem improvements and increasing knowledge of plant response to the spaceflight environment has led to the design of current plant growth systems, the latest being the Vegetable Production System (Veggie) [42, 43] and the Advanced Plant Habitat [44]. Plants can adapt to extreme environments on Earth, and model plants have been shown to grow and develop through a full life cycle in microgravity. Adequate environmental control, including forced ventilation, trace gas control, and a well-functioning system for water and nutrient delivery are required for long-term plant growth in space [3, 45].

To put this issue in perspective, the planned early Martian missions (around 500 days overall duration) will primarily focus on water recycling, atmosphere regeneration, and stockpiling of food. Due to the different orbits between Earth and Mars, the launch/return window for the trip is limited either to 30 days, or longer than 2 years (about 780 days). These relatively long space missions can only be sustained with a bioregenerative life support system. Due to the long permanence of the crew and the difficulty to transport and store a large quantity of food, it is estimated that bioregenerative life support system should provide around 80–90% of the nourishment and oxygen needed, which translates to about 40–50 m² of plant growing area needed per crew member [45]. To satisfy this requirement, permanent greenhouses and/or sizeable agricultural modules for space would need to be developed.

3.2. Plants for food in space

Growing plants in space helps solve one of the biggest issues in space travel: the supply and the price of food. Space food has evolved since 1961, when the cosmonaut German Titov became the first human to eat in space. The first foods were highly engineered, thermo-stabilized and packaged, capable of meeting the rigid requirements imposed by spacecraft

design. However, both the Apollo and the Shuttle missions demonstrated that astronauts did not consume sufficient nutrients, and determined that adequate nutrition begins with appropriate food presented to the consumer in a familiar form [46]. Accordingly, much progress has been made from the first tubed food. Today, the food for the astronauts on the ISS includes a variety of individually packaged, thermostabilized, irradiated, intermediate moisture, and natural form foods [46].

The ultimate goal of growing plants for food in space is to create a self-sustainable regenerative growth system, so plants for food could be continually grown in orbit, in Moon colonies, or on other planets. Challenges to growing plants in space are primarily in the areas of nutrient delivery, lighting, and ventilation (gas exchange). With adapted growth chambers, plant growth in space is similar to plant growth on Earth, except for some morphological traits. However, only small-scale experiments on plant growth have been performed in Earth orbit. These have not provided sufficient data on crop yield for space environments. [47]. Microgravity can reduce cell growth, alter gene expression and protein synthesis, and influence plant morphology – all aspects which critically affect plant cultivation in space. Seeds produced in space also seem to have different composition compared to seeds grown on Earth. As well as affecting the performance and nutritional content of space seeds, this could influence the flavor of plants produced in space, which might become a problem for crews reliant on plant-based diets during long space missions [47].

The theme of agriculture for space has contributed to, and benefited from, terrestrial, controlled environment agriculture; and will continue to do so into the future. For a comprehensive historical review of agricultural systems that have been developed for outer space see [4]. What started with studies on algal production in controlled environment agriculture in the 1950s in the USA and in the USSR has undergone significant improvements via NASA's Controlled Ecological Life Support Systems (CELSS) Program, Japan's Controlled Ecological Experiment Facility (CEEF), the European Space Agency's MELiSSA Project, and most recently, the Chinese Lunar Palace 1 plant factory [4, 48].

The innovative studies for space agriculture have resulted in the development of novel technologies, for both space and Earth applications. These include the use of light emitting diodes for growing crops, the demonstrations of vertical agriculture, use of hydroponic approaches for subterranean crops, crop yields that surpassed reported record field yields, the ability to quantify volatile organic compound production from whole crop stands, innovative approaches for controlling water delivery, and approaches for processing and recycling wastes back to crop production systems [4]. In addition, application of the space environment for mutagenesis and crop breeding has been suggested [26].

Recent research has focused on the possible growth of plants on the Moon and on Mars [49, 50]. In principle, it is possible to grow crops and other plant species in Martian and Lunar soil simulants, even without addition of nutrients. For the record, the Mars simulant can be obtained from a volcanic cone in Hawaii, and has a chemical composition similar to the Mars dirt that the Viking 1 lander analyzed; the Moon simulant comes from volcanic ash deposits near Flagstaff, Arizona. Beyond a Hollywood movie, experiments with 14 plant species in soils that simulate the Martian and lunar regolith suggest that future space colonizers may be able to

farm their own food using local dirt [50]. Additional research is needed to improve our understanding of the water holding capacity and other physical characteristics of the extraterrestrial soils, the availability of reactive nitrogen and other (essential) nutrients, further combined with the addition of nutrients and creating a balanced nutrient availability, and the influence of gravity, light and other conditions [50]. Further efforts should include mechanistic modeling of plant growth for better understanding of the intricate and combined physical, biochemical, and morphological phenomena involved, necessary to accurately control and predict plant growth in space.

3.3. Plants for the mental well-being of major tom

Plant in space also provide a substantial non-nutritive value; they are not just for eating or producing oxygen. Plants generally act as a form of emotional sustenance sometimes called horticultural therapy, and can mitigate the negative psychological consequences of space travel. Humans have a preference for nature scenery. Humans (especially humans in a confined space) positively react to plants, and they derive a variety of physiological benefits from exposure to plants. These include human well-being, sense of mastery of the environment, social development, health support, overcoming boredom and mental fatigue, and stress reduction and recovery [51, 52]. Studies of the potential psychological consequences of long-term exposure to conditions common to long-term isolated environments indicated that humans are less stressed and perform better in conditions that include plants and natural settings [51, 52].

The spaceflight environment induces a host of physiological, biomedical, and environmental stressors to flight crews. Long duration spaceflight has revealed a group of stressors that impact crew performance and health: hypochondria, diminished motivation and performance, impaired cognitive ability, withdrawal, impulsive behavior, hallucinations, mood swings, helplessness, depression, and anger [53]. These have spurred the emergence of areas of specialty within the behavioral sciences, including space psychology, space human factors, space habitability, space performance, and space sociology [53]. In that context, the benefit of plants as a countermeasure for difficulties experienced by humans living in isolated or extreme environments, including space travel [54]. A symbiotic relationship between plants and space travelers, including a plant garden for Major Tom, is probably a very good idea.

3.4. Case study: growing *Arabidopsis thaliana* on the International Space Station

As the International Space Station was being assembled, we designed and custom-built a novel advanced plant growth chamber for microgravity experiments [55]. The ADVanced ASTroCulture (ADVASC) was the first plant growth chamber flown on the ISS [56]. We used this chamber to grow *Arabidopsis thaliana* from seed-to-seed-to-seed (i.e., two consecutive full cycles of ontogenesis) wholly in microgravity, on the ISS. *Arabidopsis* plants were germinated, grown and maintained on the ISS prior to returning to Earth [10]. Some of these seeds were used in a subsequent experiment, to successfully produce a second (back-to-back) generation of microgravity-grown *Arabidopsis* [11].

The ADVASC plant growth unit was designed to control environment parameters including temperature, relative humidity, lighting, fluid nutrient and water delivery, and CO₂ and ethylene concentrations. Advanced control software provided control of each environmental parameter in the plant chamber, creating environmental conditions suitable for growing a wide variety of plant species. Auto-prime technologies eliminated the need for power during Space Shuttle ascent/descent, greatly relieving the shortage of Shuttle resources and the ISS crew time. Fault tolerance and recovery algorithm significantly increased overall system robustness and efficiency. Tele-science features allowed engineers and scientists to receive telemetry data, to send remote commands, and to monitor plant development status via the video images and other data (**Figure 1**).

The first flight of ADVASC provided an opportunity to study the patterns of plant growth and development, as well as seed and plant morphology in microgravity (first seed-to-seed Arabidopsis experiment on the ISS) [10]. The subsequent flight of ADVASC was used to obtain a second generation of microgravity-grown Arabidopsis plants (second seed-to-seed Arabidopsis experiment on the ISS), and to obtain fresh plant tissue for DNA microarray analysis (gene expression profiling) [11]. Since previous investigators found abnormalities in seed produced on long duration missions, we wanted to see if ADVASC's improvements in remote plant care had translated into improved seed quality. We were also interested to learn if microgravity would alter plant form and cause biochemical, cellular, and molecular changes.

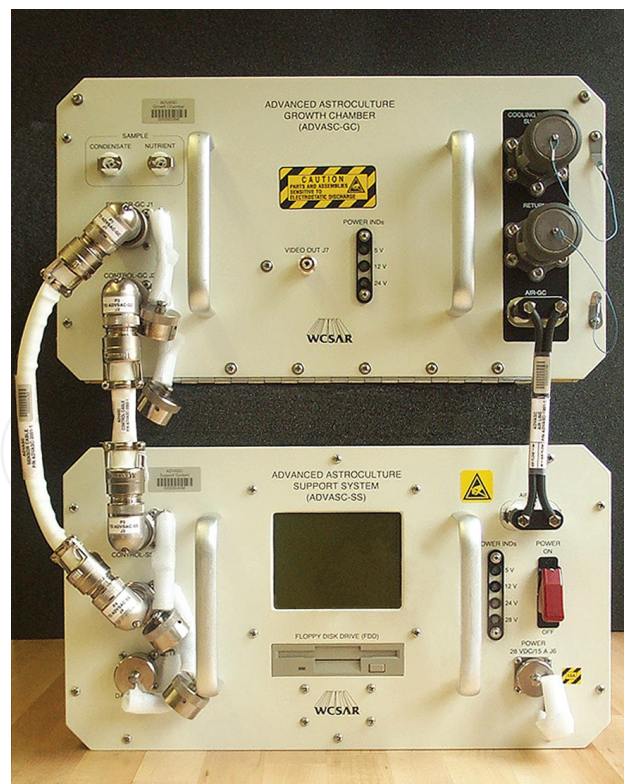


Figure 1. Advanced Astroculture (ADVASC) environmentally controlled plant growth chamber, designed for experiments on the ISS, able to support plant research for a maximum of 6 months in microgravity environment.

The first ADVASC payload with 91 *Arabidopsis thaliana* seeds planted in the root module was launched on STS-100 (ISS-6A), and returned to Earth on STS-104 (ISS-7A). During approximately 70 days in space, the experiment went through seed germination, plant development, seed formation, and seed maturity, which formed a complete life cycle. The experiment was designed to perform autonomously through the entire life cycle [10]. Post-mission analysis data shows that fully 90% of seeds germinated in space, which was similar to the 1 g-grown control plants (grown in a separate ADVASC growth chamber on Earth). Approximately 70% of seeds grew to produce siliques which contained mature seeds in space; An average of 24 siliques per plant were produced, each one containing an average of 36 seeds per silique; plants were healthy and growing normally with the exceptions of the roots and the inflorescent branches from the main stem of flowers. The directions that these organs grew were different in comparison to ground-controlled experiment, and were consistent with an apparent micro-gravity impact [10].

Plant growth and development in microgravity proceeded similarly to the ground controls that were grown under 1 g in an identical chamber [10, 11]. Morphologically, the most striking feature of space-grown *Arabidopsis* was that the secondary inflorescence branches and siliques formed nearly perpendicular angles to the inflorescence stems. The branches grew out perpendicularly to the main inflorescence stem, indicating that gravity is the key determinant of branch and silique angle, and that light has either no role or a secondary role in branch and silique orientation [10, 11]. Seed protein bodies were 55% smaller in space seed than in controls, but protein assays showed only a 9% reduction in seed protein content. Germination rates for space-produced seed were 92% indicating that the seed developed in microgravity were healthy and viable. We determined that gravity is not necessary for seed-to-seed growth of plants, though it plays a direct role in plant form, and may influence seed reserves [10, 11]. Indeed, it appears that plants undergo somewhat different growth and morphogenesis under space conditions; plant organs show automorphogenesis in space, which may be masked by gravimorphogenesis on earth, except when growing on a clinostat (**Figures 2 and 3**) [57].

Upon return of the plants to Earth, we conducted biochemical, cellular and molecular analyses. We observed a 55% reduction in protein body size; however, since the protein bodies in space-developed seed were filled and we did not observe any other signs of hypoxia such as degeneration of the embryos, deposition of starch grains or alterations in cell structures or cell numbers, we conclude that the aerial portions of the plant were not starved for oxygen. The high forced airflow rates (2–3 m/s) and accompanying ethylene removal provided by the growth chamber improved growing conditions for the aerial part of the plants when compared to the previous studies [9, 33, 39].

Root zone hypoxia could explain the reduced seed protein content. ADVASC uses passive airflow to move air through the root tray. Root zone hypoxia has been prevalent in space flight experiments [58, 59]. We used our own mix of porous arcillite matrix that is one of the favored rooting systems for space [60]. Arcillite reduces root zone hypoxia by allowing air to penetrate between the arcillite grains. Nonetheless, air movement through arcillite is restricted, especially if the spaces between arcillite grains are filled with roots, water, or both. If passive

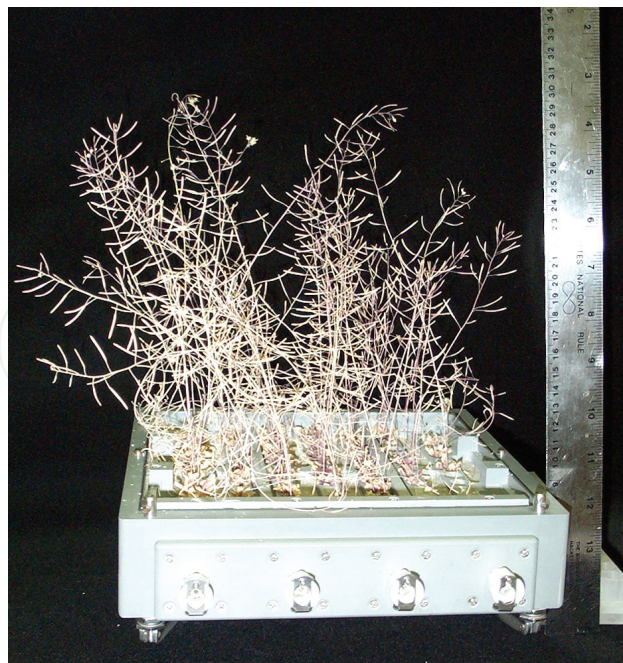


Figure 2. First plant life cycle experiment on-board the International Space Station, showing *Arabidopsis thaliana* retrieved from the ISS; these were grown in the period between ISS 6A–ISS 7A missions.



Figure 3. Expedite the Processing of Experiments to the Space Station (EXPRESS) rack 1 on the ISS is pictured on-orbit May 14, 2001, with astronaut James Voss checking ADVASC functioning. Image credit: NASA/JSC. The EXPRESS rack is a multipurpose rack system that houses and supports research aboard the space station.

airflow through the arcillite is cut off then oxygen can only reach the roots by diffusion from the air above the soil, or by the arrival of oxygenated water. Diffusion rates are negligible when the diffusion distances are more than a few millimeters [60].

Approximately 80% of the roots formed a dense mat in the top 13 mm of arcillite, while the roots of the ground control plants penetrated deeply throughout the root tray. Evapotranspiration data showed that the porous tubes in the growth chamber delivered an average of 110 mL/d of aerated water during the major growth portion of the experiment. There was not enough oxygen in this amount of water to meet the physiological needs of the roots [58]. In the absence of moisture sensor in the root tray, we had no way of knowing the relative moisture level in the root tray. An anoxic root zone in space resembles an environment similar to flooded soil on earth. Anoxia reduces nitrogen uptake by the roots therefore seed protein content is reduced. On Earth, applying fertilizer to flooded plants improves seed protein content. Because our growth chamber used an artificial soil with no native nutrient value, the plants were fertilized four times during the experiment. This may explain how the plants achieved only 82% of the normal protein content in the seed [10, 11].

This was the first report of altered branch and silique angles for space-grown plants. The reduced branch angles and perpendicular growth of the siliques in space appear to be true microgravity phenotypes. The branching pattern seen in the first spaceflight experiment [10] was replicated during the second spaceflight experiment [11], indicating that this phenotype is persistent in *Arabidopsis* development on long duration space flights. Light plays a principle role in the “upright” or light-seeking growth habit of the primary axis of many plants, and is responsible for houseplants curving towards the nearest window. On Earth, this response interacts with negative gravitropism in the shoot and requires that shoot gravitropism experiments be conducted in the dark [61]. In our spaceflight experiments the primary axis of *Arabidopsis* always grew towards the light source, supporting a central role for light in the orientation of the primary axis. The reduced branch angles and tendency of the branches to ignore or curve away from the light source in space shows that gravity plays the key role in signaling branches to curve upwards on Earth. The reduced angles that the siliques made with the stems also show that gravity has a direct role in determining the silique angles. Since *Arabidopsis* branches do not naturally curve towards the light in microgravity, light plays either a negative or a secondary role in the branch form. Spaceflight appears to initiate cellular remodeling throughout the plant, yet specific strategies of the response are distinct among specific organs of the plant. In the absence of gravity plants rely on other environmental cues to initiate the morphological responses essential to successful growth and development; the basis for that engagement lies in the differential expression of genes in an organ-specific manner [27].

We also conducted the first ever transcriptional profiling of higher plants fully grown in microgravity [11]. The gene expression data were suggestive of the presence of an abiotic stress response. However, we cautioned with respect to deriving conclusions from our gene expression profiling study, because the observed expression patterns may be at least in part induced by other interacting suboptimal environmental conditions, e.g., an anoxic root zone in space. During the second seed-to-seed experiment on the ISS (that provided plants used for transcriptional profiling), technical issues interfered with the priming of the growth chamber and its transition into steady state [11]. These may have contributed to the observed gene expression patterns.

While *Arabidopsis* plants grown in microgravity may have shown some signs of root zone hypoxia, the ADVASC growth chamber in general provided a very good environment for

growing plants on the ISS, and successfully eliminated most of the problems seen in previous plant spaceflight experiments, allowing us to discover alterations in plant form and architecture. We were thus able to successfully grow two consecutive generations of *Arabidopsis thaliana* in space, i.e., seed-to-seed-to-seed. Future experiments should be conducted to see if these alterations can be generalized across different species of plants. As well, future designs of space growth chambers (e.g., the Vegetable Production System [43] and the Advanced Plant Habitat [45]) should consider improving the root zone aeration to prevent root zone hypoxia.

4. Prospects

This is a very exciting time for space science, as the search for extraterrestrial life is one of the great intellectual enterprises of our species. At the same time, better understanding of the profound biodiversity and adaptability of life on Earth is part of the same continuum. Results from the performed space experiments were previously plagued by inconclusiveness due to the small number of experiments, small number of replicates, use of diverse flight hardware, growth conditions, limited possibilities for tissue preservation and subsequent analysis, etc. Future space experiments should therefore have standardized conditions for plant growth [3, 62]. Plus, it is the one area of space science in which you get to eat your experiment.

The theme of agriculture for space has contributed to, and benefited from, terrestrial, controlled environment agriculture; it will continue to do so into the future. The ISS ability to provide an opportunity for direct comparison of microgravity vs. 1 g (in on-board centrifuge) conditions, and for on-the-spot modification to the experiment conditions, create unprecedented advantages for plant space biology investigators. This is particularly helpful when investigators are surprised after taking a well-understood experiment on Earth and attempting to reproduce it on the ISS.

Understanding gene and protein expression is the key to unlocking the mechanisms behind microgravity-induced problems, and to finding effective countermeasures to spaceflight-induced phenotype alterations. Even though large-scale tests on growing crops for food production in microgravity are lacking, the body of acquired knowledge that there is little impediment to growing plants in microgravity, in outer space, and on other planets; even if the plants do experience some level of genotoxic stress and anatomic changes [49]. As human space exploration continues to advance, we should feel confident about our ability to grow plants on the Moon, on other planets, and on board spacecraft during long-term space missions. We still need to investigate how plants deal long-term with cosmic radiation and with the soils of other planets. We do, however, know that plants require specialized environments for growth and development in microgravity, including efficient watering and nutrient-delivery systems, precise environmental controls for temperature, humidity and air composition, and low-energy lighting. We already know how to produce such specialized growth chambers and greenhouses; we could design light absorption systems that take advantage of sunlight on the surface of planets and moons, to help us more efficiently grow plants in them.

Finally, it is not far beyond the realms of possibility that selected plant species can be genetically engineered and remotely controlled to provide food, clean air, and potable water, while at the same time acting as a source of raw materials and as small pharmaceutical factories, many miles away from Earth. Such “programmable plants” could uniquely support human missions in space by receiving and responding to remote signals for the synthesis of compounds needed yet unavailable off-the-shelf in deep space [6].

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Conflict of interest

The author declares no conflict of interest.

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