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Microgravity research in plants

A range of platforms and options allow research on plants in zero or low gravity that can yield important insights into plant physiology

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"The Brick Moon" by Edward Everett Hale, published in 1869 in the *Atlantic Monthly*, is the first science fiction story of an artificial satellite. Hale described how the low gravity on board enabled plants to evolve at high speed, providing the inhabitants of the Brick Moon with plenty of food. He already foresaw two aspects of space facilities: their dependency on sustainable food supply and their astronomical price: The Brick Moon required 12 Mio bricks and cost US$250,000, an astronomical sum by the time he wrote his story.

> *"When Charles Darwin published his first work on plant tropisms in 1880, he or his fellow researchers did not have the opportunity to perform experiments in real microgravity."*

Now, 150 years later, a human-inhabited satellite, the International Space Station (ISS), has become a reality and plant research is one of the numerous scientific disciplines that is being studied there. Recently published review articles have summarized the achievements of decades of plant research in microgravity (see Further Reading). In this article, we look at the socioeconomic aspects of this research and the impact it had in recent years and will have in the future, and at the costs equated to other, equally ambitious research projects. To compare space-based microgravity research and to highlight this unique environment, we take a look at some other facilities that are used to provide a low-gravity environment for research and at their costs and constraints.

Facilities to conduct experiments in simulated or real microgravity

When Charles Darwin published his first work on plant tropisms in 1880, he or his fellow researchers did not have the opportunity to perform experiments in real microgravity. Instead, scientists had to rely for a long time on changing or randomizing the gravity vector by turning plants from a vertical into a horizontal position (gravitropic stimulus), or by using centrifuges (hypergravity). Over time, more sophisticated tools, such as clinostats and random positioning machines, were developed. However, these platforms only provide what we call simulated microgravity and not real microgravity, and, depending on the biological system that is studied, the interpretation of results might be challenging.

> *"Only free fall can achieve real microgravity."*

Only free fall can achieve real microgravity. A drop capsule in free fall, a plane during a parabolic flight, a sounding rocket, or a satellite creates a centrifugal force, which compensates the gravitational pull of the Earth with residual acceleration forces in the range of 10^{-2}–10^{-6} g. These experimental platforms differ in their time of microgravity provided, the mode of operating the hardware, the quality of microgravity, and the price (Fig 1, Table 1).

> *"[The ISS] is by far the most expensive low-gravity platform to perform experiments, but [. . .] is a unique experimental platform for microgravity research."*

The free fall in the vacuum tube of a drop tower generates up to 5 s of exposure to high-quality microgravity or up to 10 s when a catapult is used to propel the experiment into the drop tower. With 6,000–10,000€ per drop, it is one of the cheapest microgravity platforms and the turnaround time of 2–3 drops per day widely surpasses that of other platforms. The major constraints are the short time and the up to 50 g of landing acceleration, which does not make drop towers suitable for every experimental hardware. For experiments with biological samples, such as plants, the drop tower has become more attractive in recent years as fast molecular readouts, such as phosphoproteomics and secondary messenger signaling that play a role during the first seconds of a microgravity response, came more into focus.

The other ground-based facility is airplane parabolic flights that provide up to 30 s of microgravity. In Europe, the largest aircraft for parabolic flight research is the Airbus A310 ZERO-G, operated by Novespace by the order of the European Space Agency (ESA), and the French

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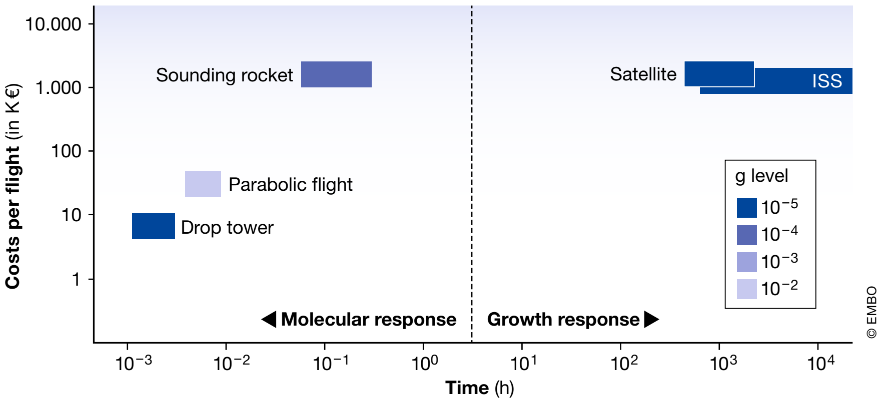

Figure 1. Low-gravity costs, magnitude, and duration for the various low-gravity platforms.

(CNES) and German (DLR) space agencies. The plane regularly flies 31 parabolas with microgravity of up to 22 s per parabola.

Three features make parabolic flights unique. One is the ability to produce partial g-levels, as experienced on the Moon (0.16 g) or Mars (0.38 g). The second is that, unlike the drop tower, experiments can be manually operated by crew members, allowing to change experimental parameters during the flight. Third, the parabolic flight is the only microgravity platform that allows biomedical experiments with human subjects. The deceleration of 1.8 g is also much lower compared to the drop tower. A constraint is the low level of microgravity of 10^{-2} g and the start acceleration of 1.8 g before and after each parabola. Thus, all biological samples have already been exposed to both hyper- and microgravity after the first parabola, which makes the results more challenging to interpret.

The ISS

Only space enables long-term microgravity experiments (Fig 1). The first plant biology experiment in space took place in 1946 on board a repurposed V2 rocket launched by NASA. It has maize seeds on board, but no plant growth took place during the experiment. Sounding rockets, such as the European MAXUS rocket, provide up to 13 min of microgravity with a level of 10^{-4} g and recovery of the rockets within 1–2 h allows for downstream sample analysis. Onboard centrifuges are often used as an inflight 1 g control to compensate for vibrations and start acceleration. Indeed, start accelerations of 6–12 g are a significant constraint of these experiments, as plants were shown to quickly respond to hypergravity of 5 g with changes in gene expression [1].

Growth and developmental responses to alterations in gravity, as measured on Earth using gravitropism assays, require hours of observation on a phenotypical level (Fig 1). Such long-term developmental changes have therefore to be studied in low Earth orbit on board of satellites or human-tended space laboratories, such as in the ISS, the Salyut and Kosmos space stations, Skylab, Space Shuttles, the Russian space station MIR, and, more recently, the Chinese space lab Tiangong. Among these, the ISS is the largest and longest inhabited space station with a continuous human habitation since November 2000. It is by far the most expensive low-gravity platform to perform experiments, but since it offers months or even years of experimental time, crew operation of the experimental, and download opportunities, it is a unique experimental platform for microgravity research.

The presence of heavy masses in our solar system, notably the planets, does not allow for a real zero-gravity environment. Even on the ISS, the gravity level is still 92% of the gravity level on Earth—after all, the ISS orbits the Earth at an altitude of merely 350 km. It is the continuous free fall of the station toward Earth at a speed of around 28,000 km/h that creates low-gravity levels of 10^{-2}–10^{-6} g.

The facilities for plant growth experimentation on board the ISS have significantly grown over time. BIOLAB, the European Modular Cultivation System (EMCS), KUBIK (ESA incubator with centrifuge platform), and the Cell Biology Experiment Facility (CBEF) are now available to conduct experiments. During the past decade, it became evident that many of the earlier results were hampered by engineering constraints and suboptimal plant growth conditions [2]. All hardware platforms nowadays therefore include 1 g reference centrifuges to

Table 1. Microgravity test facilities.

Test facility	Time	Operation	Constraints	g-level	Costs (€)/flight[a]	Flights
Drop tower	5–9 s	A	- Brief flight time. - 5–50 g landing acceleration. - 30 g start acceleration in catapult mode.	10^{-4}–10^{-8}	6–10 K	2–3 per day
Parabolic flights	15–30 s	A, M, B	- Low level of microgravity	10^{-2}–10^{-3}	30 K	18 p.a.
Sounding rockets	4–13 min	A, M	- Increased launch acceleration up to 6 g	10^{-2}–10^{-4}	1–2 M	2 p.a.
Recoverable satellites	Up to 3 month	A	- High launch accelerations, long preparation phase	10^{-3}–10^{-5}	> 1 M	< 1 p.a.
International Space Station (ISS)	Up to years	A, M, B	- Long preparation phase	10^{-2}–10^{-5}	> 1 M	Several p.a.

A = automatic, M = manual, B = manned.
[a] Costs of space experiments depend on size and weight and can thus only be estimated roughly.

distinguish between non-gravity and space- and growth-dependent effects [2]. Modern technologies are readily adapted to allow leading-edge experimentation, such as FLUMIAS, a structured illumination microscope for live-cell imaging in space built by German industry on a DLR contract.

> *“. . . satellites offer an excellent quality of microgravity that is not affected by docking maneuvers or crew movement.”*

One of the main advantages of the ISS is the possibility to send seeds to the station and start growing plants on board. This way, artifacts from prior hypergravity stress during the rocket launch are mitigated. However, the hypergravity phase during the rocket flight and the long time between the loading of the rocket and the actual experiment on board are still major constraints. Moreover, limited crew time often requires automation of experiments, which is readily achievable for gene or protein expression studies, but presents a challenge for microscopy. In addition, the movement of the crew and docking maneuvers create microgravity changes on board; as a result, the onboard microgravity is, over time, less constant than on ground-based facilities such as the drop tower or other space-based platforms, such as satellites or sounding rockets.

Indeed, satellites offer an excellent quality of microgravity that is not affected by docking maneuvers or crew movement. Experiments can be conducted from weeks to months, limited only by the lifetime of the biological samples. Then, sizes of satellites used for plant experiments range from the Eu:CROPIS with a weight of 230 kg and outside dimension of 1 × 1 m that can house two greenhouses down to CubeSats with 10 × 10 × 10 cm size and 1 kg weight. Eu:CROPIS was recently launched with tomato plants and Euglena algae on board. Standardization, off-the-shelf components, and an emphasis on simplicity and low costs allow affordable missions with project timelines of typically 9–24 months from inception to launch. The downsides of satellites are the limited space and the fact that the hardware is often not reusable and that temperature control is challenging in the smaller satellites.

Funding of microgravity research on Earth and in space

The funding system for experiments in microgravity is entirely different from regular research funding. The majority of the budget goes to maintaining the existing microgravity research facilities: drop towers, parabolic flight aircraft, and foremost the ISS. This is unique in the field of plant research and more comparable to large-scale physics project, such as the Large Hadron Collider (LHC) at CERN. Both research platforms were built in the same time frame: 1998–2013 (ISS) and 1998–2008 (CERN). Both are multinational research projects with 16 and 111 member countries, respectively. Building the ISS costs about US$100 billion, with a European share of around €8 billion, while the LHC costs nearly ten times less. Europe’s annual obligation to NASA for running the ISS is equivalent to about €150 million and makes up only a small part of the total annual costs of €5 billion.

> *“With experimental costs beyond €1 million, the question is whether the use of the ISS as a research platform makes sense from an economic point of view.”*

With experimental costs beyond €1 million, the question is whether the use of the ISS as a research platform makes sense from an economic point of view. One option for cutting costs in space missions is to replace human astronauts with robotic systems. This idea attracted some interest recently after comments by British Astronomer Lord Martin Rees and the head of the Russian Space agency, Dmitry Rogozin, who advocated robotic missions. In fact, robots pioneered space before humans and have already been on the Moon and Mars. Recent exploratory space missions, such as New Horizon, also used robotic systems.

We are only slowly replacing robots with astronauts in space for good reasons. Astronauts are more flexible, can respond to unexpected situations, and are much better qualified to install and calibrate new experimental hardware. Yet, current advances in robotic systems operated by artificial intelligence may eventually improve the flexibility of technical systems as such that they perform close to humans. However, the main reason we send astronauts into space is to evaluate how the human body reacts to space conditions, including blood pressure, immune system, salt regulation, osteoporosis, and aging. Astronauts these days are very much involved in medical experiments often including their own body. Here, no replacement is possible.

> *“Plant research in microgravity has contributed significantly to our understanding of plant gravity perception, signal transduction and the mechanisms of gravity-oriented growth. . .”*

Outsourcing upload, download, and operation of experiments to commercial platforms have significantly reduced the costs of research on board the ISS. Companies such as Space Tango and NanoRacks offer standardized, fully automated rigs that can run multiple experiments simultaneously. The platforms provide standard connectors for power and data and offer interactive operation from Earth and download capabilities for images and data. The experimental hardware has a standard form factor, further lowering developmental costs.

Insights from space-based experiments

Gravity masks certain phenomena on Earth either directly or by adding a layer of complexity as it influences secondary processes such as convection currents, fluid dynamics, and sedimentation. Removing gravity allows studying these phenomena. For example, the lack of gravity and convective forces improves the formation of protein crystals, which helps to determine protein structures for drug development [3]. Additionally, the study of fluids and materials in microgravity allows investigating the influence of other forces such as fluid tension, thereby leading to the development of smaller and more capable electronic devices, new metal alloys, and ceramics with unique properties [4].

Low-gravity research on plants is central for fundamental exploration of plant behavior and the use of plants in regenerative life support systems that are crucial for long-term exploratory space missions. Plant research in microgravity has contributed significantly to our understanding of plant gravity perception, signal transduction, and the mechanisms of gravity-oriented growth on a molecular, cellular, and physiological level [2]. We learned that plants can respond to minimal gravitational forces. Lentil seedling roots grown on board the ISS reacted to a threshold acceleration between 0 and 0.002 g [5]. This changed our understanding of their gravity response: For a long time, it was believed that much higher g-forces were necessary to elicit a response in plants.

Significant progress has also been achieved in the area of gravity-affected gene expression and translation, the identification of gravity-related genes, gravity effects on metabolic processes, wound healing, and stress responses [2]. Moreover, the removal of gravity allows for the discovery of mechanisms masked by gravity but essential for plant growth on Earth. For example, a red-light-mediated positive phototropic response was discovered in *Arabidopsis* roots in microgravity experiments [6]. It demonstrated for the first time that *Arabidopsis* roots display two phototropic responses: the previously characterized blue-light-dependent negative phototropism and the red-light-induced positive response. Last but not least, low-gravity environments also allowed to dissect whether specific movements and plant growth patterns are innate or dependent on gravity. Sunflower seedlings still displayed rotational growth and circumnutation in the absence of gravity, following an endogenous program [7]. Thus, neither a gravitational nor an inertial g-force was an absolute requirement for initiation or continuation of circumnutation. More importantly, this study showed that, on average, circumnutation was significantly more vigorous in real microgravity than on Earth-based clinostats, showing that rotation on a clinostat is not the functional equivalent of weightlessness.

These three selected examples document that microgravity research has great potential both for understanding fundamental physiological responses and for applications in plant breeding.

Potential applications for space exploration

In contrast to early explorers on Earth who could live of the land, astronauts have to rely on regular water, food, and air supplies from Earth. Consequently, life support systems that can produce food and recycle oxygen and water—in other words, plants—are needed either for long-term space missions or for establishing a human foothold on Moon or Mars. Experiments on board the ISS have shown that plants in space principally retain their inherent growth patterns even if they show changes in cell wall compositions,

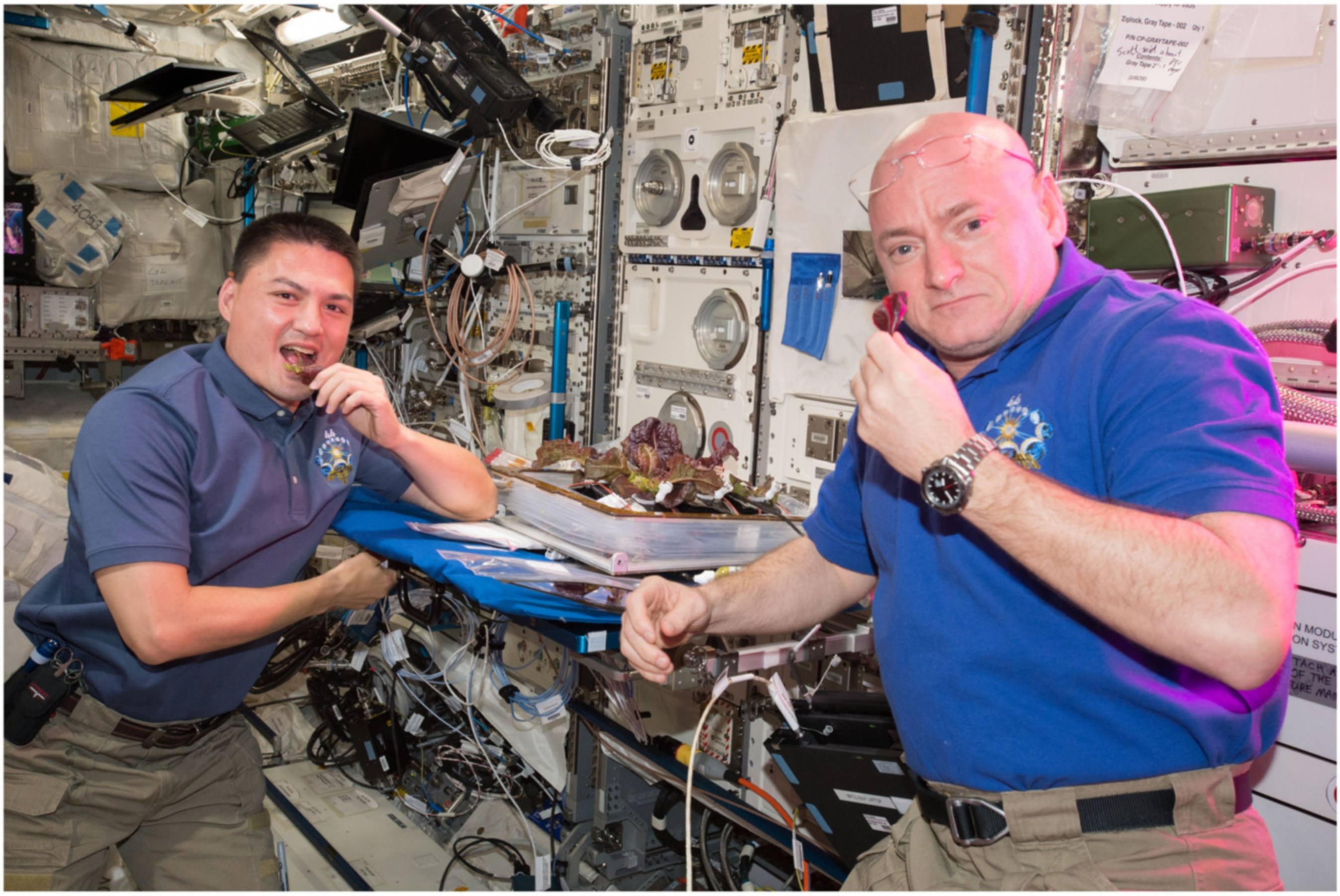

Figure 2. Tasting of the first salad that was grown, harvested, and eaten on board the ISS. With permissions by NASA.

morphology, and cell cycle [2]. The results from early experiments have also led to the development of optimized plant growth hardware, and plants for human consumption have successfully been grown and tested by ISS astronauts (Fig 2).

> *“. . . ground-based studies have great importance not only for developing the best hardware, but also to pinpoint the exact biological question that can be answered by a sounding rocket or an ISS experiment. . .”*

Each crew member on the ISS uses about 800 g of food per meal per day. A trip to Mars would require something between 6 and 8 months on an optimized trajectory. Given the alignment of the planets, another 18 months will have to be spent on Mars before the return flight, which adds up to 3 years for a Mars mission. Each astronaut would need 2.6 tons of food. Transporting this amount of food is not practical, and a combined approach of preprocessed packaged food with a long shelf life and crop growth on Mars is the currently favored solution. Fresh food also offers more variety and has tremendous psychological benefits for the crew.

Growing food on the Mars surface would present a significant challenge. On the one hand, Martian soil is much better suited for plant growth than Hollywood would make us believe. It contains all the required minerals. However, perchlorates enriched on the Martian surface would negatively affect plant growth and thus would have to be removed, for instance, by washing the soil. Mixed greens have shown to prosper in Martian soil, while potatoes did not do so well owing to the soil density [8].

On the other hand, the surface temperature is −55°C, the atmospheric pressure is < 1% of the pressure on Earth, and the atmosphere is 99% CO_2. The Mars surface receives less than half the amount of sunlight the Earth does, not accounting for dust storms, and it receives a higher dose of UV light due to a missing ozone layer. Greenhouses on Mars will have to be airtight, highly pressurized, and heated. It appears to be an excellent long-term perspective but might prove too ambitious for the first missions.

Box: Further reading

Science fiction
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Historical work on plant tropisms
Darwin CR (1880) *The power of movement in plants*. London: John Murray
Plant Space Biology
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Sabbatini M (ESA), Sentse N (Space consultants) (2014) *ESA USEr GUidE to Low GrAvity PLAtformS - European Space Agency*
Ground-based facilities
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Concerning the supply with oxygen, a recent experiment in the Chinese closed ecosystem Lunar Palace 1 in Beijing showed that a crew composed of three members needed close to 2 kg O_2 per day [9]. According to this and similar studies from NASA, this can be produced by a growth area of 70–75 m^2 of crops [9]. For upcoming Mars missions, various scenarios exist with typical crew sizes between three and six astronauts. A recent proposal estimates that supporting a crew of six with exclusively natural light would require up to 24 greenhouses with growth areas of 90 m^2 each [10]. Artificial light may help to reduce the growth area down to three greenhouses.

Critical considerations for experiments in space

Not only the costs but also the difficulties in developing experimental hardware for plant cultivation, treatment, fixation, and analysis are factors that determine the choice of platform. The time needed for payload development and integration varies significantly between individual low-gravity platforms: drop towers (6 months), parabolic plane flights (6 months), sounding rockets (2 years), and ISS (5 years). The potential for yielding meaningful results crucially depends on these preparations. Thus, ground-based studies have great importance not only for developing the best hardware, but also to pinpoint the exact biological question that can be answered by a sounding rocket or an ISS experiment with limited biological replicates under challenging conditions. Ground-based low-gravity platforms can complement space-based approaches by helping to understand the biological systems, building improved hardware, testing new concepts, and verifying results obtained in space, but they can never entirely replace space-based platforms. Moreover, we should never forget that many technical advances developed for particular missions in science have subsequently been used in laboratory research or led to technological developments not foreseen.

The ISS is so far the only available low-gravity research platform of its kind. Its unique properties are long-term microgravity, crew operation, and state-of-the-art research hardware, which makes it essential for both fundamental and applied plant microgravity research.

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Conflict of interest

The authors declare that they have no conflict of interest.

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