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SPACE EXPLORERS NEED TO BE SPACE FARMERS

What we know and what we need to know about plant growth in space

F. Javier Medina

Space exploration will require life support systems, in which plants can provide nutrients, oxygen, moisture, and psychological well-being and eliminate wastes. In alien environments, plants must adapt to a different gravity force, even the zero gravity of spaceflight. Under these conditions, essential cellular and molecular features related to plant development are altered and changes in gene expression occur. In lunar gravity, the effects are comparable to microgravity, while the gravity of Mars produces milder alterations. Nevertheless, it has been possible to develop and reproduce plants in space. Current research seeks to identify signals replacing gravity for driving plant growth, such as light. Counteracting gravitational stress will help in enabling agriculture in extraterrestrial habitats.

Keywords: plant biology, International Space Station (ISS), microgravity, root meristem, gene expression.

INTRODUCTION

On 10 August 2015, the image of three crewmembers of the International Space Station (ISS) eating a lettuce, grown and harvested onboard, impacted mass media all over the world. «It was one small bite or man, one giant leap for #NASAVEGGIE and our #JourneytoMars. #YearInSpace», wrote the astronaut Scott Kelly in Twitter (StationCDRKelly, 2015) below the videoclip showing the salad snack (seen also in Figure 1A). He had become the first space farmer.

This event was an outstanding outcome of the project Vegetable Production System («Veggie»), a plant growth facility that had been capable of producing a small crop of a salad-type plant to provide the crew with a safe source of fresh food of good quality in terms of taste and nutrition. In addition, the culture served as a relaxing activity, very different from the routine procedures for the maintenance of the Station, and for their own physiological wellbeing. Veggie provides lighting and nutrient delivery, but utilizes the cabin environment for temperature control and gas exchange (Figure 1B).

«Plant space biology is quickly progressing, especially since the assembly and operation of the International Space Station»

«The farther and longer humans go away from Earth, the greater the need to be able to grow plants for food, atmosphere recycling and psychological benefits», said Gioia Massa (NASA, 2015), Veggie's payload scientist. «I think that plant systems will become important components of any long-duration exploration scenario», she concluded. These statements expressed by Dr. Massa are shared by all parties involved in the coming enterprises of space exploration, from scientists to managers and decision-makers of the most important space agencies worldwide. However, does this mean that all the obstacles for the successful and continuous growth of plants in space have been removed?

The answer, from the perspective of plant biology research, is «no», or, at least, «not yet».

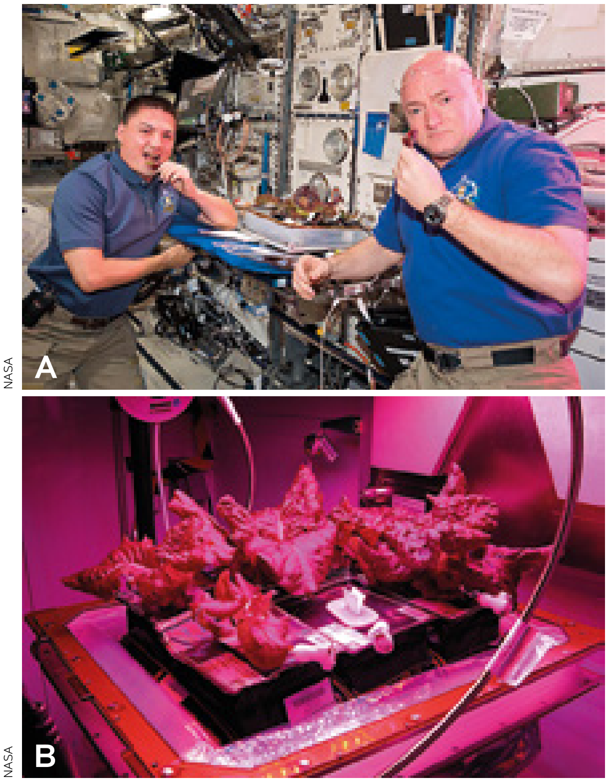


Figure 1. **A)** NASA astronauts Scott Kelly (right) and Kjell Lindgren (left) snack on freshly harvested space-grown lettuce as part of the Veg-01 experiment. **B)** Facilities of NASA's «Vegetable Production System» project («Veggie») at the International Space Station. The picture shows the Veg-01 nursery containing red romaine lettuce seedlings.

Plant biologists believe that the initial results of Veggie, followed by other recent achievements, open new questions and challenges to the scientific work. It is true that a higher plant did grow in space until an adult stage showing apparently the same shape, features, and composition as they display on Earth. Nevertheless, we do not know how that plant overcame the cellular and molecular alterations caused by the exposure to the space environment, repeatedly reported (Herranz & Medina, 2014). This paradox was already discovered by Marco and coworkers (Marco et al., 2003) after pioneering experiments with the fruitfly *Drosophila* revealing that, apparently, development was completed normally, despite changes found in cellular and molecular parameters. Finding the keys to solve this paradox is one of the most exciting current challenges of space biology research.

«Space travel is a crucial milestone for plant history: for the first time plants face the absence of gravity»

THE SPACE ENVIRONMENT: RADIATION AND GRAVITY

Many objects are orbiting around the Earth, Moon, Mars, or beyond, within an environment that, from the terrestrial perspective, is harsh and dangerous for the life. Certainly, the only orbiting object harboring terrestrial life outside the Earth is the ISS (and the spaceships related to its maintenance).

The Earth is the only planet of the Solar System endowed with an atmosphere compatible with the existence of living beings. Both the Earth atmosphere and the magnetosphere protect the life from the hostile conditions of the outer space, including vacuum, radiation, and temperature. Any initiative of space exploration, both within the spaceship and in any sort of habitat to be established in the Moon or Mars, must incorporate shielding against these factors and should create a replica of the terrestrial atmosphere in its interior.

Special attention should be paid to cosmic radiation, as a major environmental factor to consider during space missions. Radiations cause cellular damage that can be either lethal, or affect the physiology of tissues and organs. Exposure to high doses of radiation is a major danger for space explorers and this was taken into account during the construction of spacecrafts, which implemented protective shields. However, exposure to lower doses of more penetrating radiations, which pass across shields and are cumulative, induces a stressed state on organisms. This is a risk for longer-term missions, such as travelling to Mars.

The other major factor of space environment is gravity alteration. Gravity is the force of attraction existing between two objects. According to Newton's law of universal gravitation, the force is directly proportional to the product of their masses and inversely proportional to the square of the distance between their centers. Thus, the acceleration of gravity is 9.8 m/s² (1g) in the surface of the Earth, and it is reduced to 9.0 m/s² (0.9g) in a low Earth orbit, such as the distance to the ISS (around 400 km from Earth). A reduction to $10^{-2}g$ could only be reached at a distance of 200,000 km from Earth, without considering the influence of other objects.

Then, why the astronauts in ISS, or in spacecrafts orbiting the Earth, experience «zero-gravity»? This exists in the ISS because of its orbiting state, which is an endless free fall around the Earth. An orbiting object is pulled towards the center of our planet by gravity, but the lateral movement, at high speed, prevents it to fall and keeps it at a constant distance. The result of the centrifugal force produced by the orbit and the gravity force towards the Earth is «weightlessness», or «microgravity», or more colloquially «zero-gravity» (effective gravity $<10^{-3}g$). This is the status in a space vehicle orbiting the Earth. However, at the surface of the Moon or Mars, the gravity force depends on the mass of the satellite or planet, being 0.17*g* in the case of the Moon, and 0.38*g* in the case of Mars.

The change of gravity existing in the space environment compared to Earth is especially important for space exploration. In practice, counteracting physically this change could only be possible by continuous centrifugation, which has not been considered until now an efficient and affordable option. Therefore, the strategy is to know its effects on living beings and try to mitigate the physiological alterations by using biological mechanisms eventually leading to the adaptation of living beings to the new environment.

«Special attention should be paid to cosmic radiation, as a major environmental factor to consider during space missions»

GRAVITY HAS MODELLED THE PLANET EARTH, THE LIFE, AND THE PLANTS

Among environmental factors influencing living beings, gravity is the only one with a constant presence on Earth throughout the entire history of life. Not only the biological evolution, but also the geological processes driving the formation of the planet Earth have occurred in the presence of a constant gravity vector, which is indeed still operating nowadays. Morphology and functions of terrestrial living beings are greatly influenced by gravity and different organs, systems, and processes aimed to detect and respond to gravity, as well as to use gravity in the benefit of the biological functions, can be found throughout the Earth biodiversity.

In the particular case of plants, a crucial milestone in plant evolution was the colonization of land by aquatic plant ancestors that occurred ca. 400 million years ago. For their emergence from the sea and their growth on mainland, plants had to develop a rigid body capable of resisting the force of gravity and to keep the plant standing. Success was based on the elaboration of protective walls affecting extended areas of the whole plant body, such as lignin layers, which also served to prevent desiccation. Then, plants differentiated specialized aerial organs to maximize the exposure of leaves to the sunlight and the efficiency of photosynthesis, and underground roots and root hairs to capture water and mineral salts from the soil. Plants used gravity for this differentiation and developed gravitropism, to direct the growth according to the gravity vector. Thus, roots exhibit positive gravitropism growing towards the center of the Earth, whereas stems have negative gravitropism, growing towards the sunlight.

In this context, space travel is another crucial milestone for plant history: for the first time, plants face an environmental condition – microgravity, which is absent from their memory, not only ontogenetic but also phylogenetic. The first space experiments, more than 50 years ago, showed that plants could survive and grow in space, although alterations were soon reported (Perbal, 2001). Results were sometimes confuse, in most cases due to deficiencies in experimental setup and in devices used to germinate seeds and grow plants. Improvements in culture facilities such as the case of Veggie, have allowed us to conclude that microgravity itself does not prevent plant growth and reproduction. Thus, the pending question is to know the mechanisms used by plants to overcome alterations and reach a functional adaptation. Any possible answer to this question should take into account that plants have developed during evolution a high plasticity to adapt to changing environmental conditions, due to their sessile condition. This plasticity is based on the existence of meristematic tissues and on the high redundancy of the plant genomes and the gene families existing in them.

MICROGRAVITY CONSEQUENCES FOR PLANTS

The root meristem is an essential tissue for plant development and stress response. It is a permanent reserve of undifferentiated totipotent cells, which generate differentiated cells for plant development. The function of meristematic cells is a continuous cycle of growth and division – the cell cycle (Figure 2). Mitotic cell division produces two daughter cells, which grow through interphase in the G1 and G2 phases, which are separated

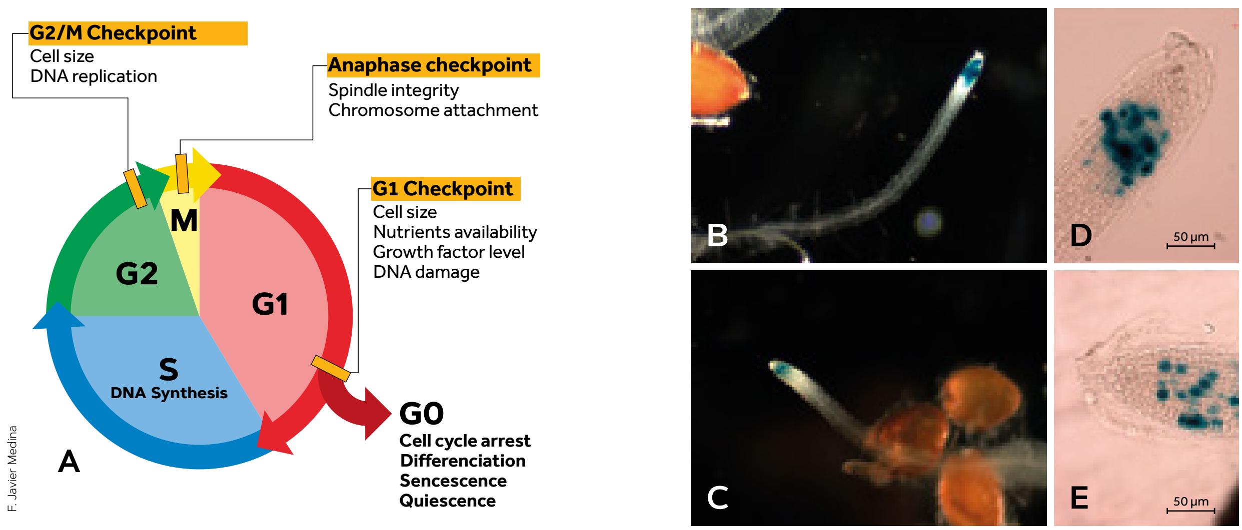


Figure 2. **A)** Schematic representation of the cell cycle. The four phases are represented (G1, S, G2 and M – mitosis), as well as the checkpoints, in which regulatory processes take place. In each checkpoint, the specific processes and parameters to be checked are indicated. When cells cease their progression throughout the cell cycle, they enter in the G0 phase. The fate of G0 cells is variable and the major alternatives are indicated. **B, C)** Visualization in the root meristem of the expression of cyclin B1, a protein involved in the G2/M checkpoint in the cycle, detected by means of the genetic reporter construction *cycB1:GUS*, observed with a binocular stereoscope. Seedlings were grown under control 1*g* conditions (B) or under simulated microgravity (C). **D, E)** Visualization of the cyclin B1 expression as in the precedent images, but observed with the light microscope. The expression of the cell cycle regulator is much decreased in simulated microgravity.

by S phase, characterized by DNA replication (Figure 2). Cell growth in cycling meristematic cells is not merely an increase of cell size, but an increase of the protein content of the cell, enabling further cell division (Perrot-Rechenmann, 2010). Since proteins are synthesized in ribosomes, the nucleolus and ribosome biogenesis are faithful markers of meristematic cell growth. In general, meristematic cell function stands on a strict coordination between the rates of cell proliferation and cell growth, termed «meristematic competence» (see Herranz & Medina, 2014).

The «Root» experiment, the first European experiment on plant biology carried out in the ISS, showed a striking increase of the cell proliferation rate in the root meristems of space samples, accompanied by a decrease of the rate of cell growth, expressed as production of pre-ribosomal precursors in the nucleolus (Matía et al., 2010). Previous pioneering space experiments had already observed alterations in certain parameters of the cell cycle (Perbal, 2001). Complementary assays performed in microgravity simulation facilities on ground confirmed this uncoupling of cell proliferation and ribosome biogenesis induced by microgravity. More specifically, alterations in cell cycle regulation have been observed producing the shortening of the G2 phase, the most active in ribosome production and, consequently, in mitosis anticipation (Kamal et al., 2019). Thus, the absence of gravity as an environmental condition causes a noticeable stress on the plant due to the loss of meristematic competence (Herranz & Medina, 2014) (Figure 2).

Meristematic competence is physiologically connected with gravity sensing by means of the phytohormone auxin. Gravity alteration produces changes in the lateral balance of auxin in the root producing skews and bends, whose consequence is the loss of orientation and the alteration of gravitropism (Gadalla et al., 2018). Moreover, auxin is a major driver of the meristematic activity by regulating cell growth and cell proliferation (Perrot-Rechenmann, 2010). The existence of additional cellular gravity sensors, probably located in the cell wall, and of intracellular signal transduction mechanisms, has been postulated (Herranz & Medina, 2014).

Apart from the cellular effects, the microgravity environment causes gene reprogramming in plants. The absence of a «memory» of weightlessness throughout plant evolution raises the key question on whether the organism responds to the absence of this factor by developing an entirely new mechanism, or using general, non-specialized mechanisms of response. Transcriptomic studies have collectively shown a complex response in plants.

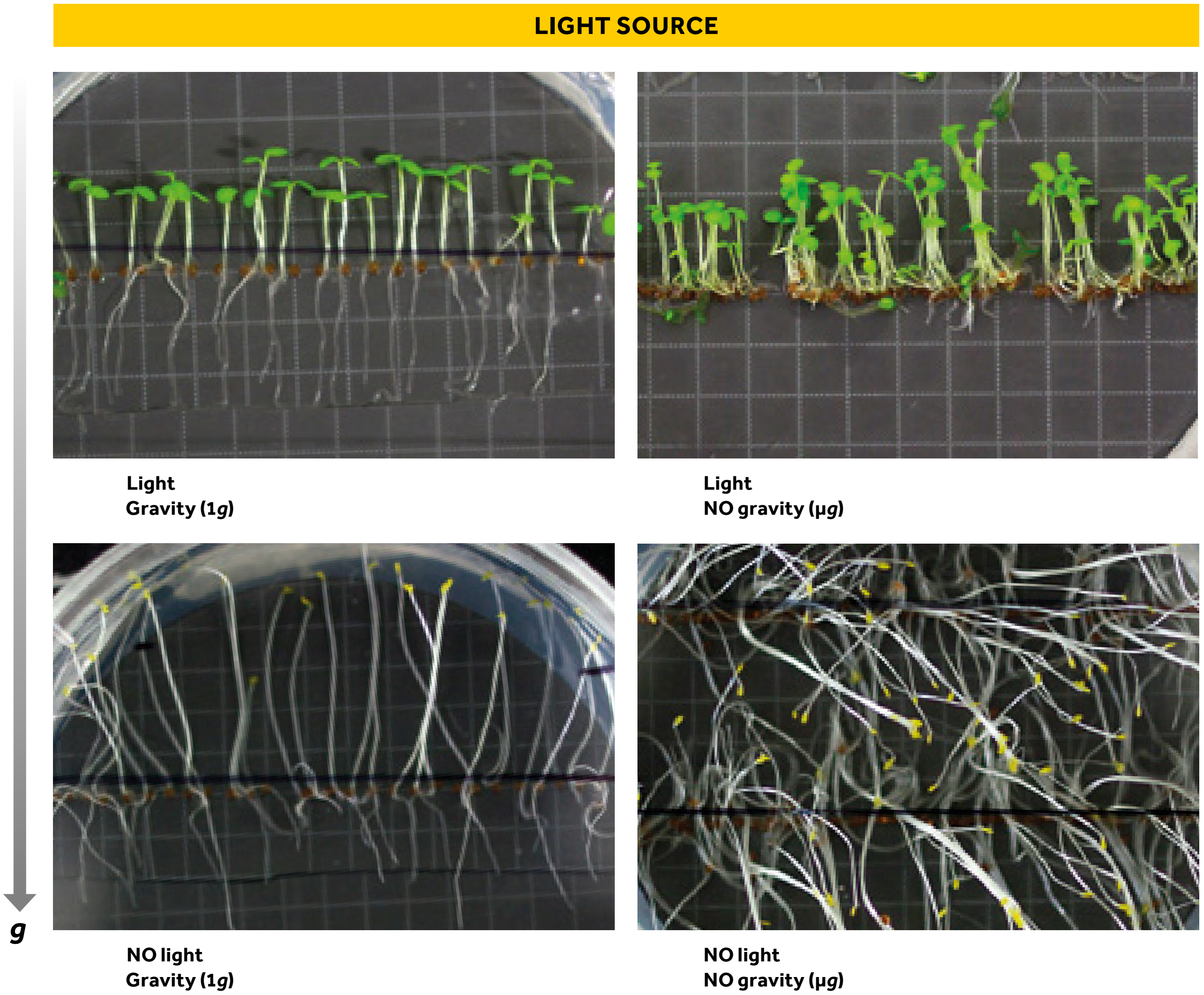


Figure 3. Effects of light and gravity on the orientation of stems (hypocotyls) and roots in young seedlings. The different possibilities of the combination of the two environmental tropistic factors are shown. Note the different effects on the two organs of the seedling. The position of the light source and the direction of the gravity vector (*g*) (when applicable) are indicated.

Specific genes of response to gravity alteration have not been found up to now, but, instead, genes known to participate in general mechanisms of response to abiotic stresses have been shown to modify their expression in the microgravity environment (see Paul et al., 2017, and references therein).

THE ROLE OF LIGHT

Despite the physiological changes caused by the space environment, adult plants and flowers develop in space with apparent normality. Therefore, the practical key question is to identify and understand the strategies triggered by the plant in order to counteract the survival problems associated with the space environment and microgravity. The key word in this important research objective is *adaptation mechanism(s)*. What are these mechanisms and how and when are they established?

The adaptation and survival of plants in space could greatly benefit from the substitution of gravity by another external cue, which could play the same or a similar role in driving plant growth and development as gravity does on Earth. Light is a good candidate, since it is indeed a tropistic stimulus. Phototropism complements gravitropism with the objective of optimizing the efficiency of the capture of nutrients (Figure 3). In addition, illumination, especially by red light, is sensed by phytochromes to produce changes in the regulation of auxin responsive genes and many growth coordinators (Vandenbrink et al., 2014).

In this context, it would be interesting to know to what extent light can act as a signal capable of counteracting the effects of the lack of gravity. For this purpose, the series of experiments termed the «Seedling Growth Project» was conducted in the ISS (Vandenbrink et al., 2014), constituted by three consecutive experiments performed respectively in 2013, 2014, and 2017. The project was the result of the cooperation of NASA and the European Space Agency (ESA), using a European incubator combined with an American culture chamber for seed germination and growth of seedlings. Different collections of mutants of the model plant species *Arabidopsis thaliana*, affecting phytochromes, nucleolar proteins, and auxin responsive genes, were used. Seeds germinated in flight and grew for six days under different regimes of illumination and gravity. In addition to microgravity, seedlings were subjected to different levels of gravity between 0*g* and 1*g*, including the Moon and Mars gravity levels, which were produced by a centrifuge installed in the incubator (Figure 4).

Although some of the analyses are still in progress, new phototropic responses to blue light in space have already been identified, which complement previous findings. Furthermore, a positive effect of red light in counteracting the stress caused by microgravity on cell growth and proliferation in the root meristem has been found (Valbuena et al., 2018), which was confirmed by a global transcriptomic study. Therefore, it appears that (red) light could be a factor promoting adaptation of plants to microgravity and it can be used to mitigate gravitational stress for the culture of plants in space.

«The change of gravity existing in the space environment compared to Earth is a challenge for plant survival»

GROWTH OF PLANTS IN THE FRACTIONAL GRAVITY OF MOON AND MARS

Plant culture for space exploration is needed not only on board of orbiting spaceships, characterized by a microgravity environment, but also in the more or less permanent settlements of humans in alien planets or satellites (the Moon and Mars are the primary targets, for obvious reasons).

The harsh and hostile environment of the Moon and Mars is a major constraint for the project and implementation of any «greenhouse». The concept of *terraforming* of a planet or moon is emerging from science fiction (literature, cinema) to real science to designate the human process of modifying its atmosphere, temperature, surface topography, or ecology to be similar to the environment of Earth to make it habitable by terrestrial life. Interestingly, experiments performed with soil simulants have shown that plant growth and development are compatible with the surface of Mars and Moon.

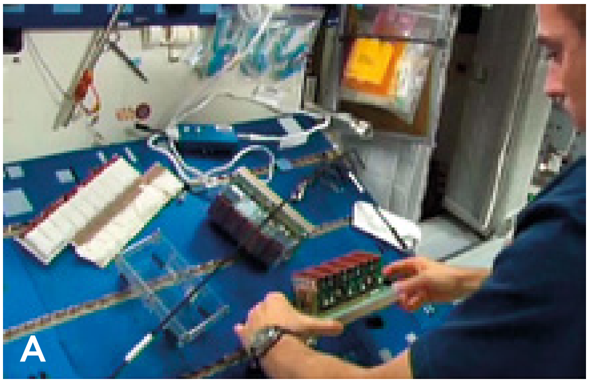
Again, gravity appears as a permanent and unavoidable factor. As previously said, Moon gravity is 0.17*g* and Mars gravity is 0.38*g*. These belong to the so-called partial-*g* levels, indicating that the gravity vector is a fraction of the Earth magnitude. These partial-*g* levels were simulated on Earth by implementing technological solutions that were validated biologically by growing *Arabidopsis thaliana* seedlings in the modified devices. Seedlings grown under the simulated Moon gravity level appeared severely affected in the measured physiological parameters, with an intensity even stronger than the effects recorded under simulated microgravity, whereas the Mars gravity level only produced a mild disturbance of the balance between cell growth and proliferation, with parameters similar to those obtained from control 1*g* samples (Manzano et al., 2018).

Interestingly, the simulated Moon gravity could not orient the growth of seedlings, but, under the Mars gravity, seedlings exhibited a conspicuous gravitropism. In turn, the results obtained in space, using the centrifuge to produce a wide spectrum of partial-*g* values, followed the same trend as obtained on ground with simulation devices. Thus, it appears that the gravity of Mars will not be a major obstacle for the culture of plants.

FUTURE PROSPECTS

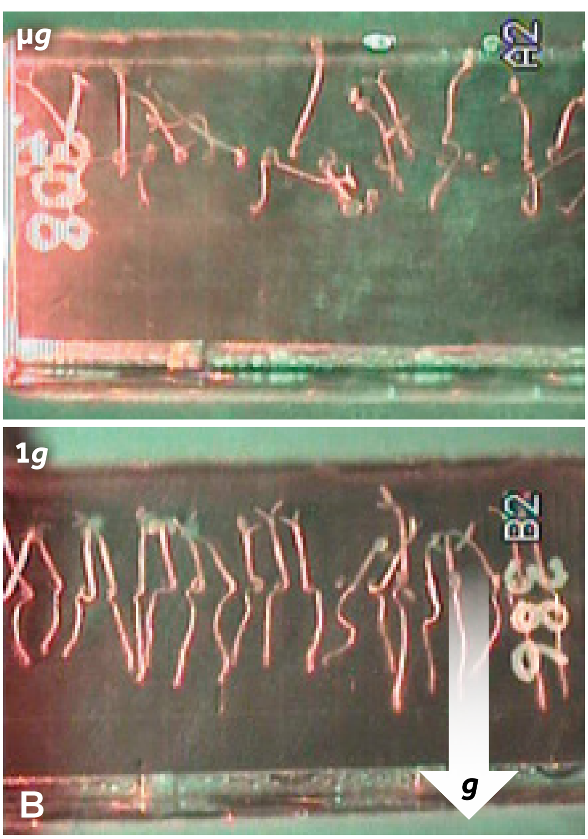
The ESA web page dedicated to plant biology research in space[1] underlines the importance of space agriculture and emphasizes the need of research in this discipline, starting with this paragraph: «Cultivating plants for food was a significant step in the history of mankind. Growing plants for food in space and on other planets will be necessary for exploration of our Universe».

1 https://www.esa.int/Science_Exploration/Human_and_Robotic_Exploration/Research/Plants



NASA TV

Figure 4. Images of the «Seedling Growth» space experiment, taken in the International Space Station. **A**) An astronaut has extracted from the incubator the experimental containers and the culture chambers in which seedlings have grown, and is performing the operations needed for the accurate preservation of samples to make possible their transport to the Earth and their further analysis in the laboratory. **B**) Images of seedlings grown in space according to the experimental protocols. μ*g* represents microgravity and *1g* represents control ground gravity. In this case, 1*g* seedlings grew in an on-board centrifuge rotating at this angular speed. The orientation of the growth of seedlings greatly differs in the two cases. The direction of the gravity vector (*g*) in the control experiment is indicated.



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The establishment of transient or permanent human settlements in the Moon and Mars is becoming realistic after repeated overall success of unmanned robotic missions. Despite some technological, ethical, and political questions that still need to be answered, the pathway for this enterprise is open. By looking at mass media and social networks, we perceive that space research for space exploration attracts much interest from people. Specifically, the search for extraterrestrial life and the survival and adaptation of terrestrial living beings in alien environments greatly excite the curiosity of citizens. Moreover, space exploration is a powerful booster for general scientific and technological progress and for the public awareness of its importance.

«Light can act as a signal capable of counteracting the effects of the lack of gravity»

Whereas it is becoming increasingly clearer that humans need to be space explorers, it is equally clear that space explorers need to be space farmers. The Ridley Scott's movie *The Martian* (2015) highlighted this need in a scene that shows in a well-documented manner the type of challenges and solutions that arise in the space exploration related to the culture and use of plants. Obviously, this scene (as well as the entire movie) belongs to the category of science fiction, but our expectations, based upon actual scientific experiments, are not too far from the stories shown in the movie.

Plant space biology is quickly progressing, especially since the assembly and operation of ISS. Despite constraints and limitations that still affect to space experimentation, we have generated in the last ten years a knowledge that is progressively enabling us to produce space crops capable of feeding space explorers. For example, we now know quite well the gene reprogramming induced in plants when they adapt to the space environment.

A few topics could be listed here among the most relevant and urgent challenges for the near future in this field. Regarding gene expression, we need to define the significant proportion of «unknown function» genes affected by microgravity that has been found in transcriptomic studies. In plant physiology, the full discrimination of the mechanisms of adaptation, especially looking at the role played by light in driving the plant growth in the absence of gravity, is still pending, as well as the precise function of auxin in signal transduction in the different scenarios of altered environmental stimuli. The role of microgravity on senescence and oxidative processes is of fundamental importance and it has been insufficiently experimented. Finally (and the list is not exhaustive) we need to eventually jump from the model system of *Arabidopsis* to real crop species as the material for our experiments.

> «What would you take to space? […] Oxygen, water and food […], your favorite books, music, electronic devices, something to write, to draw… You should also take medicines.»

On these intriguing and exciting questions, a group of Spanish students of a high school in Navarra has developed a project on the use of plants in space for the production and purification of recombinant proteins. These proteins could be, for example, medicines whose expiry date exceeds the duration of the space mission, or nutrients that are not found naturally in plants. The project called BioGalaxy,[2] prepared by the students with the support of the Pamplona Planetarium and the Institute of Agrobiotechnology, placed second in the International iGEM Competition. The future is open and nobody knows the end of the story.

«Plant culture for space exploration is needed not only on board of orbiting spaceships, but also in the more or less permanent settlements of humans in alien planets or satellites»

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[2] **http://2018.igem.org/Team:Navarra_BG**