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Growing Plants in Human Space Exploration Enterprises

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Abstract. The coming enterprises of space exploration by humans, e.g. the future colonization of the Moon and Mars, will require the utilization of plants as key components of Bioregenerative Life Support Systems. The space environment is very different from the Earth environment in many factors. Many of these adverse factors can be counteracted in spaceships, or in Martian or Lunar settlements, but the living beings must adapt to grow and survive in microgravity. The Earth gravity has remained constant in magnitude and direction throughout the entire history of our planet, including biological evolution. Gravity establishes the direction of plant growth through the process called gravitropism and this orientation is essential for the normal function of roots, stems and leaves, assuring the adequate nutrition of the plant. The phytohormone auxin is known to play a main role in gravitropism. Auxin is also a major regulator of plant development, since this hormone is ultimately responsible of the maintenance of meristematic cells, which are totipotent cells, continuously engaged in the cell cycle, and are the suppliers of differentiated cells for plant development. Meristematic competence is the balance between cell growth and cell proliferation occurring in meristematic cells. A major effect of the microgravity environment is the disruption of meristematic competence, comprising the increase of the proliferation rate and the decrease of the growth rate, estimated through the rate of production of ribosomes, the cellular factories of proteins. Microgravity also induces a noticeable reprogramming of gene expression. In meristematic cells, genes driving the cell cycle regulation are affected. In general, the systems responsible of the plant defense against abiotic stresses and the energy/redox systems are major targets of the gene reprogramming. Noticeably, no specific genes related to gravity alteration have been identified, although a significant proportion of altered genes encode unknown functions. Despite these cellular and molecular alterations, plants are capable of surviving, developing until the adult stage, and even reproducing under microgravity conditions. This means that plants indeed adapt to this environment, although the mechanisms of adaptation are currently unknown. A major challenge of current research is to identify environmental cues that may replace gravity in driving growth and development. Light can be one of these cues and understanding the role of light, as a countermeasure for gravitational stress, will contribute to the success of the culture of plants in extraterrestrial habitats.

1 Introduction

"The earth is blue". This was the astonishing message received on 12 th April 1961 by the Ground Control of the "Vostok 1" spacecraft, led by Yuri A. Gagarin, the first human leaving the Earth's atmosphere. The USSR had won the race to put a man into space. Gagarin orbited the planet and returned to Earth one hour and 49 minutes after launch, landing safely. "How wonderful. It is amazing", he reported. The world had changed. Only eight years later, on 20 th July 1969, Neil A. Arm-

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strong, commander of the "Apollo XI" mission became the first person to step foot on the surface of the Moon. That race had been won by Americans. "That's one small step for [a] man, one giant leap for mankind", was the historical sentence recorded from him once in the surface of our satellite. Nowadays, fifty years after Armstrong's epic achievement, space exploration by humans is commonly recognized as a highly exciting and attractive challenge and a powerful booster for scientific and technological progress in order to improve the human life on Earth. This is true despite some criticisms (minority, but significant) that question the high costs that it entails [1]. The establishment of permanent settlements in the Moon and Mars is becoming a realistic venture day by day. Both ESA and NASA, and more recently the agencies from growing economies in Asian countries, are working to promote a manned mission to Mars after a decade of succesful rover explorations [2]. These objectives require the implementation of a complex system of Life Support for space explorers, capable of supplying the elements necessary for sustaining their life (oxygen, food, moisture ...) and of removing their waste products. The system needs to be bioregenerative, i.e. the components need to self-regenerate, without the addition of new elements brought from the Earth, and energetically efficient, only using the power sources available in space. Plants are a candidate to occupy a key position in these Bioregenerative Life Support Systems (BLSS). They indeed are being used in all the initiatives tested up to now, such as MELiSSA [3, 4]. There is no doubt that plants must accompany humans in space exploration ventures acting as supports of human life as well as a source of psychological wellbeing for space travelers. The achievement of a true "space agriculture" is a fundamental objective of this global enterprise and the advances in this task are already producing substantial benefits for the efficiency and sustainability of the terrestrial agriculture. Actually, the existence of "Martian greenhouses", installed on the surface of alien planets, is an image that does not anymore appear strange to our minds, although it is still a matter of science fiction Growing plants successfully in space requires a full understanding of the biological mechanisms of response and adaptation to the conditions of the space environment. This environment is very different from the one where the biological evolution, and particularly plant evolution, has occurred and produced the biodiversity currently existing in our planet.

2 The Space Environment: Radiation, Gravity

For many reasons, the space environment is incompatible with the survival of the terrestrial life. Actually, the Earth is the only planet of the Solar System surrounded by an atmosphere, whose chemical composition and physical properties allow the existence of living beings. Any initiative of space exploration involving living beings, and specifically humans, must incorporate a protection or shielding within the spaceship or human habitat against the hostile factors of the space environment, among which radiation, deep vacuum and space debris. Specifically, cosmic radiation is known to be the cause of cellular damage that can be either lethal or affect the physiology of tissues and organs at different levels. It is known that the level of shielding currently existing for spacecrafts, including the International Space Station (ISS), does not totally prevent the exposure to low doses of penetrating radiations. Due to a cumulative effect, this could represent a risk for long-term missions, such as those comprising the travel to Mars. Another major factor of space environment is gravity alteration. According to the Newton's law of universal gravitation, at the surface of the Moon or Mars, the gravity force depends on the mass of the satellite or planet, being, in relation to the Earth's gravity, 0.17g in the case of the Moon, and 0.38g in the case of Mars. However, in a space vehicle orbiting the Earth, the resultant 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). The change of gravity existing in the space environment compared to Earth is especially important for space exploration. Trying to counteract this change with physical countermeasures is neither efficient, nor affordable (continuous centrifugation is not an option for living beings). Therefore, the useful strategy is to know its effects on living beings and using biological methods to mitigate the physiological alterations eventually leading to the adaptation of living beings to the environment of altered gravity.

3 Gravity, Life and Plants: Gravitropism

The existence of the gravity vector is a physical factor, basic and permanent in the Earth from the formation

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of the planet. Gravity has shaped our world and it has been constantly present during the whole process of biological evolution over the Earth, so that it has exerted an influence in modelling the life we know now. Gravity is indeed a weak force by itself, but it is responsible for physical processes such as buoyancy, sedimentation, convection and hydrostatic pressure, which are at the basis of the chemical (including biochemical) reactions and are decisive to make our world such as it is. The fact that living beings are capable of sensing the gravitational force means that they are capable of transforming a physical stimulus into a biological process. To know the mechanisms responsible for this transformation is an exciting and intriguing question that is only beginning to be discerned, still in a few cellular systems. It is of particular interest to investigate the role played by gravity (and the modifications induced by its alteration) on the processes of growth, shaping and development in plants [5, 6]. Plants are probably the living beings in which the influence of gravity is more evidently detected, since the plant growth depends on root and stem orientation according to the gravity vector, by a mechanism known as gravitropism, whose mechanism was discovered, in general terms, more than a century ago, but it is still under research to discern its molecular details.

According to the classical starch-statolith hypothesis of gravity sensing, the mechano- stimulus of gravity is sensed in the root in certain cells of the columella, located at the tip, called statocytes, which contain starch granules (statoliths) that sediment according to the gravity vector, producing the polarization of the cell. A cascade of signals transmits the physical information derived from statolith sedimentation to the cells of the elongation zone (Figure 1). The movement of statoliths along the endoplasmic reticulum (ER) exerts pressure on the ER membranes and on the plasma membrane and cause membrane deformation capable of opening membrane-localized mechanosensitive calcium channels [7]. An additional interaction of statoliths with the actin microfilament cytoskeleton causing tensions in microfilaments with the same consequence of the opening of calcium channels in membranes (tensegrity model) was also proposed [8]. Anyway, and increase in the levels of calcium triggers the gravitropic signal transduction pathway. The consequence of the transduction of the mechanosignal is the reorientation of auxin efflux carriers and subsequent redistribution of auxin streams in the root cap and the root as a whole. An interplay between calcium levels and auxin transport has been reported [9]. Under normal environmental conditions, and in absence of reorientations of the plant with respect to the gravity vector, auxin is synthesized in young shoot tissues and transported acropetally into the root tip, where an additional amount of newly synthesized auxin is added. Then, auxin is transported basipetally, throughout peripheral tissues of the root, to the elongation zone, where it regulates cellular elongation [10]. The transport is carried out by means of specific auxin efflux and influx carrier proteins, among which is the auxin influx facilitator AUX1, whose loss of function produces an agravitropic phenotype [11], and the PIN family of auxin efflux facilitators [12]. When the plant growth is not affected by any reorientation with respect to the gravity vector, auxin distributes evenly in the meristematic and elongation zones of the root, and this grows straight and downwards. However, if the position of the root is altered, involving a reorientation with respect to the gravity vector (e.g., by turning the root to a horizontal position), a lateral gradient of auxin is established in the root. The localization of efflux carrier proteins shows an asymmetric distribution, as it was reported for PIN3 protein [13] . This induces a faster growth and/or elongation of the cellular layers of the upper side of the root with respect to the lower layers and, eventually, the root curvature [14]. These data are in agreement with the classical theory of Cholodny-Went who stated for the first time in 1928 that the asymmetric growth was due to the lateral distribution of auxin. The mechanism of the alteration of auxin polar transport is well understood, if this is consequent to a definite change in the gravity vector with respect to the growth direction. However, our knowledge is more limited as to the effects of the total suppression of gravitropic stimulus, such as it occurs in weightlessness, or under microgravity conditions, e.g. on board of spaceships.

Growth in microgravity (real or simulated) results in the loss of a definite orientation of the root growth (Figure 2). Furthermore, a substantial inhibition of the auxin polar transport was reported in early experiments [15]. Otherwise, when auxin transport was experimentally inhibited, the gravitropic response was suppressed [16]. Root morphogenesis and changes in the auxin levels were measured in the root tips of rapeseed seedlings grown in a clinostat. Under these conditions, secondary roots were initiated earlier so that the biomass of the root system was 30plants than in the vertical control. The determination of the levels of auxin showed that, after few days of growth on the

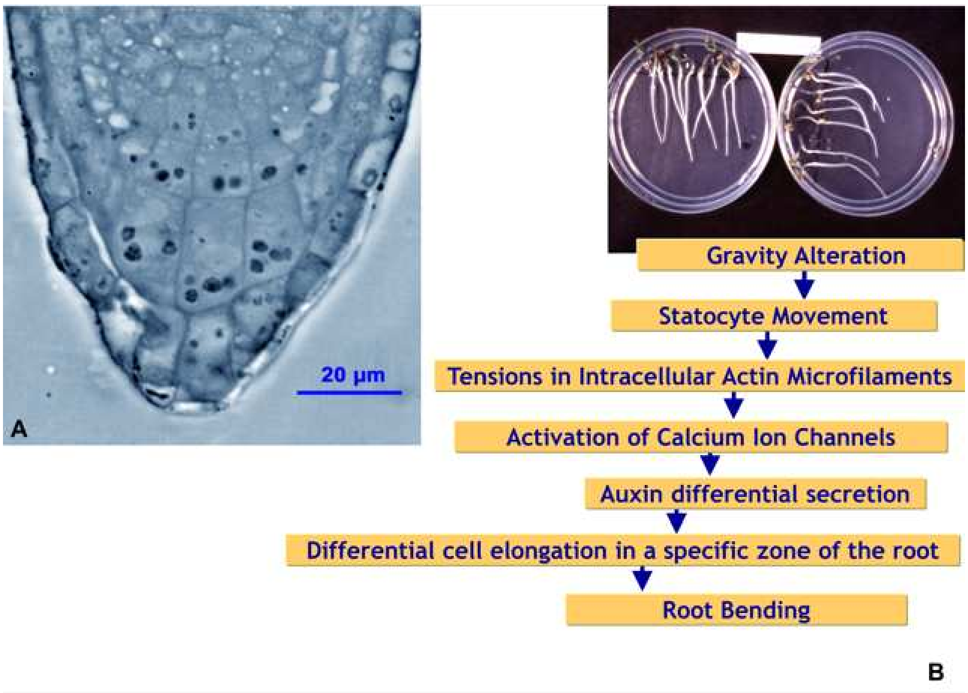

Figure 1. *Gravitropism. A) Longitudinal section of a root tip of* Arabidopsis thaliana *observed under the phase contrast light microscope. Collumella cells show amyloplasts (statoliths) clustered in the bottom part of the cell due to their sedimentation following the gravity vector. B) Sequence of physiological events in the root initiated by the perception of a change in the direction of the gravity vector with respect to the direction of the root growth, leading to the change of root orientation (root bending).*

clinostat, the increased length of the primary root coincided with an increase in auxin contents [17]. In a study using different systems of microgravity simulation, namely magnetic levitation and the Random Positioning Machine (RPM), our laboratory reported that the pattern of auxin distribution in the root tip under simulated microgravity corresponds to the inhibition of the auxin polar transport. Visualization with the reporter gene construction DR5::GUS showed an accumulation of the auxin signal in an extended area of the root tip [18]. In a more recent experiment performed in space, the distribution of auxin in the root was shown to display a "vertical" pattern, similar to the pattern of roots grown under control ground gravity. Since the seedlings grew in the absence of any physical force that could influence the direction of their growth, as gravity does in the Earth, the roots showed a complete disorientation, including numerous bends, coils and skews [19]. This would mean that the auxin transport through the root and the balance of auxin distribution in the root would be independent from gravity sensing. Actually, more research is necessary to explain these findings in the context of the current models of relationships between statolith movement, auxin transport and root growth direction. However, significant differences between spaceflight and ground controls were observed in the cytokinin distribution in the root tip, suggesting an involvement of this hormone in gravity-related developmental events of the root [19]. It can be concluded that, although the basic cellular mechanisms involved in gravitropism are relatively well known, many details remain to be elucidated, requiring a concerted work in the fields of genomics, proteomics, cell biology, biochemistry and physiology.

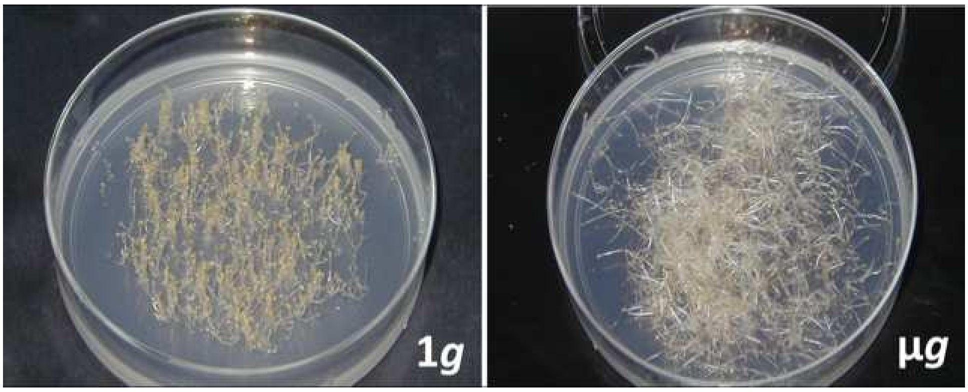

FIGURE 2. *Orientation of seedlings after growing them for 4 days in darkness under control ground gravity (1g), or under simulated microgravity (µg). Ground gravity produces seedlings all of them perfectly aligned in the direction of the gravity vector. Microgravity produces a mess of seedlings without any orientation for roots or hypocotyls.*

4 Plant Development, Proliferating Cells and Microgravity. The Root Meristem

Meristematic tissues are composed by undifferentiated, totipotent cells with a high capacity of cell proliferation and cell growth, capable of producing any specialized tissue at any time in the life of the plant. Apical meristems are located at the ends of the body of the plant, in roots and shoots (Figure 3). In general, meristems are the source of cells for plant development, such that this process greatly depends on the balance between cell proliferation and cell differentiation that exists in meristems [20]. Environmental conditions modulate meristematic activities, directly or indirectly, at different levels of regulation [21]. In a relatively small number of experiments performed in space and in ground-based devices of simulated microgravity it has been specifically approached the influence of environmental gravity on meristematic cell functions [see [22]]. In early pioneering studies on plant space biology, changes in mitotic index of lentil roots grown in microgravity were reported [23, 24]. The interpretation was difficult, since changes in this parameter may be due to different types of alterations throughout the cell cycle, in interphase and mitosis. In further experiments in real and simulated microgravity, comprising analyses of higher complexity, the progression of cell cycle appeared modified, even though the cell elongation did not appear affected. The densitometric analysis of nuclear DNA content of meristematic cells from roots grown in microgravity showed changes in the proportion of cells in different cell cycle phases, suggesting that the regulation of the cell cycle progression is modulated by gravity [25, 26]. The first European experiment on plant biology on board the ISS revealed that one of the most relevant effects of altered gravity is the disruption of the meristematic competence in cells of the root apical meristem [27, 28]. Under microgravity conditions, cell proliferation and cell growth appear uncoupled, losing their coordinated progress, which is characteristic of these cells under normal ground gravity conditions. Cell proliferation rate is increased and cell growth, estimated by the rate of production of ribosomes, the cellular protein factories, is depleted. Further experiments performed on ground-based facilities for microgravity simulation, including sequential sampling at different growth times and the analysis of gene expression, have confirmed the uncoupling of cell proliferation and ribosome biogenesis caused by altered gravity, showing that the weightlessness environment is a stress condition, specifically for plant proliferating cells. The effects of the gravitational stress are detected from the very beginning of germination, in two-day-old seedlings [29, 30]. The enhanced cell proliferation rate is not accompanied by an increase in the levels of cyclin B1, a regulator of the G2/M transition, as would be the normal in ground gravity, but, on the contrary, these levels appear depleted. Since cyclin B1 is synthesized in the G2 phase of the cell cycle, a shortening of G2 phase is compatible with these observations. The causes of this shortening could be found in a failure or malfunction of the cell size checkpoint, which immediately precedes mito-

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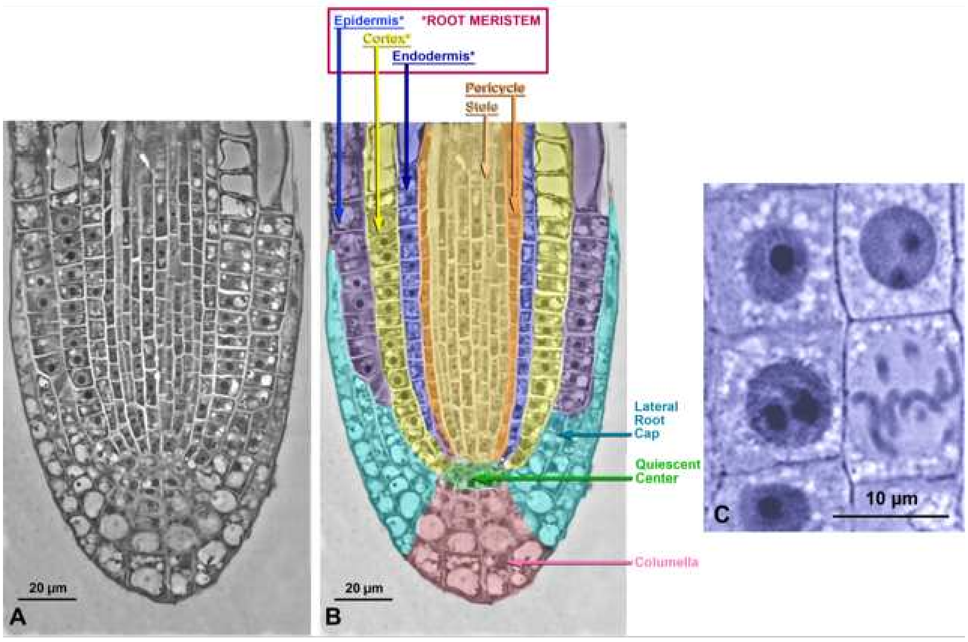

Figure 3. *The Root Meristem. A) Longitudinal section of the distal part of a root of* Arabidopsis thaliana *observed under the phase contrast light microscope. B) The same image showing the different layers and types of cells. The root meristem is composed by epidermis, cortex and endodermis. C) Meristematic cells are nearly cubic cells, showing a prominent nucleus and nucleolus, centrally located, and small vacuoles. Mitotic figures are frequently observed in microscopical sections.*

sis [31]. At the same time, a lower cellular growth was observed, since ribosomes were produced at a lower rate, indicating a reduced protein biosynthesis. This depletion of ribosome biogenesis was already observed in meristematic root cells grown in simulated microgravity [32, 33], but it was not put in relation to other cellular processes. The shortening of the G2 period of the cell cycle, as the result of the effect of microgravity on cell cycle regulation, is also compatible with these findings, since the G2 period is known to be the most active in ribosome production [34]. The observations and interpretations made on root meristems from seedlings have been confirmed and demonstrated in studies performed on in vitro cultured cells grown in simulated microgravity. Flow cytometry analysis provided evidence for an accelerated cell cycle in cells grown in the Random Positioning Machine (RPM). The final acceleration was the result of a shorter G2 phase and a slightly longer G1 phase. Analysis of gene expression showed a general downregulation of genes involved in the G2/M transition checkpoint and upregulation of many genes controlling the G1/S transition. This is accompanied by downregulation of significant genes regulating ribosome biogenesis and by depletion of the levels of nucleolin and fibrillarin, nucleolar proteins considered as markers of this process. In addition, a general depletion of the nuclear transcription was detected, accompanied by an increase in chromatin condensation, which was related to changes in enzymes involved in epigenetic regulation of gene expression [35, 36, 37]. All these experiments have contributed to identify the effects of the lack of detection of a gravity vector on meristematic cells. Now, we are in a better position to understand the limitations for plant growth and development that may occur in space. However, we still need to elucidate the factor triggering the cascade of functional events that eventually result in the alteration of meristematic cell proliferation and growth and in the disruption of meristematic competence. According to previously published data, the change in the hormonal signaling pathway mediated by the auxin polar transport could be a possible candidate to play this triggering role. In fact, the phytohormone auxin is a chief controller of the balance between cell proliferation and cell differentiation existing in meristems, which is the basis of the fundamental involvement of the meristematic tissue in plant development [20]. Moreover, auxin influences multiple aspects of plant growth and development, including the regulation of cell cycle progression and the coordination between cell growth and cell division [38]. From a more general viewpoint, auxin regulates the connection between stimuli perceived by the plant and the cellular responses to them [39]. As stated above, mechanical sensing of a gravity change by columella cells is converted into a relocation of PIN proteins, finally resulting in changes in the auxin gradient in the root which lead to the change of orientation of the root growth [13]. Therefore, everything points to auxin as the key mediator between altered gravity sensing and the observed effects on root meristematic cells [40].

5 Plant Genomes. Genomic / Transcriptomic Effects of Gravity Alteration

Since microgravity is a novel and very unusual environmental factor for organisms, the genome lacks particular gene sets evolved to respond to it. However, plant genomes have a reserve of plasticity that enables them to respond to unforeseen environmental conditions, such as gravity alteration. In general, this plasticity is necessary due to the sessile character of plants that immobilizes them to the ground and disables them to escape to another place if the environmental conditions became adverse. One of the sources of genome plasticity is gene redundancy, a feature much more extended in plants than in any other taxonomic group. Plants have undergone extensive duplications (up to four times) of large genomic regions, producing a large amount of duplicated genes. As known examples, specifically related to the system of cell proliferation and cell growth, the genome of the plant model species *Arabidopsis thaliana* contains two copies of the gene coding for nucleolin, a protein actively involved in different steps of the biogenesis of ribosomes. Under normal unstressed environmental conditions, only one of the genes is expressed (as it occurs in animals and yeast). The expression of the second gene only takes place under special conditions (e.g. stress) and/or in specific developmental stages [41]. Moreover, multiple cyclins and CDKs involved in cell proliferation control exhibit redundant mechanisms for producing the same effect in cell cycle checkpoints [42]. In addition to gene redundancy, quicker mechanisms like transposable elements or epigenetic processes, such as DNA methylation operate under environmental stress [43]. In particular, plants exposed to spaceflight exhibit an increased frequency of both permanent (transposable elements) and non-permanent (epigenetic) alterations [44, 45]. Epigenetic alterations were also found in plant-cultured cells exposed to simulated microgravity [35, 36]. A number of studies with *Arabidopsis* gene arrays and RNA microsequencing have identified a wide range of genes whose expression changes in response to gravitropic stimulation, simulated microgravity, or hypergravity. [46, 47, 48, 49, 50]. Many of the alterations were part of a general stress response, but some changes can be specifically attributed to the gravity alteration and indicate a possible interplay between gravitropic and other mechanical responses [51, 52]. The work performed in our laboratory using *Arabidopsis* cultured cells in simulated microgravity facilities was focused on the transcriptional changes affecting proliferating cells and, specifically, to the effects of altered gravity on the different phases of the cell cycle. Synchronic and asynchronic cells incubated in the RPM, as microgravity simulator, showed a general depletion of their transcriptional status, confirming previous results obtained with in situ methods. Different phases of the cell cycle appeared differentially affected. This was the consequence of a differential effect of the gravitational stress on the two cell cycle checkpoints, namely G2/M, which precedes cell division (the most sensitive), and G1/S, which checks the cell status before DNA replication. Thus, the cell cycle regulation is deeply reorganized under the altered gravity environment, which results in serious consequences on plant growth and development. With regards to the affected functions, our results are in line with previous reports, as to the implications of general mechanisms of stress response, such as the heat shock complex and energy/redox processes, in addition to genes coding for membrane and cell wall proteins, probably related to mechanisms of graviresistance. It is interesting to remark that 30% of the upregulated genes and 40% of the downregulated genes correspond to genes of unknown function [37]. The alteration of gravity not only changes the pattern of gene expression, but the gene reprogramming that occurs in the microgravity environment also alters the way by which plants respond to other environmental factors [50, 37]. This indicates that the transcriptome needs to be finely tuned in a global way to counteract multiple gravity- related parameters of the environment.

6 The Response to Gravitational Stress: Plant Adaptation and Its Mechanisms

From the precedent sections of this article, we can get the unequivocal conclusion that plants undergo severe changes when they sense gravity alterations. The lack of perception of gravity induces the loss of gravitropism, producing a redistribution of hormones and intense changes in cellular and molecular processes. We have also mentioned the disruption of meristematic competence, the modification of the cell cycle regulation and an intense reprogramming of the pattern of gene expression, affecting all different tissues and cellular types of the entire plant, throughout all developmental stages. If these physiological changes would be persistent, they would result in a serious compromise to the viability of the plant and, in any case, they would dramatically affect plant development. However, a few experiments have proven that it is possible to complete the full life cycle of plants (seed-to-seed) in space. Using advanced growth chambers that, in general, provided a well-regulated environment for growing plants in microgravity on the ISS, fertile adult plants have been produced from seeds germinated in space and seeds obtained from these plants have been, in turn, germinated. As examples of these experiments, we can mention the “Advanced Astroculture (ADVASC)” experiment, consisting of two successive experiments carried out in 2001-2002. The seeds obtained from the first experiment were successfully germinated in the second one. The authors reported morphological changes in branch and silique orientation of *Arabidopsis* plants relative to stem and small differences in protein content (91% of control) and germination rate (92% of control) of spaceflight grown plants [53]. A second experiment was the Japanese “Space Seed”, carried out in the “Kibo” module of ISS in 2009 [54]. Using one of the latest and most advanced facilities to growth plants in the ISS, the so- called “Vegetable Production System” (Veggie) the image of three astronauts snacking on the first space-grown edible plant, a freshly harvested red romaine lettuce, went around the world and saturated the Internet social networks. Apart from the social interest, widely disseminated by mass media, this achievement had an undoubted interest from a strictly scientific perspective: an adult specimen of a higher plant species (a sporophyte, in botanical terms) had been successfully grown in space [55]. A few months later, a beautiful Zinnia flower produced in the Veggie facility on board ISS was photographed and, again, the picture went around the world transmitted by Internet social networks (`https://www.nasa.gov/image-feature/first-flower-grown-in-space- stations-veggie-facility`). Together with the beauty of the image, the scientists saw in it the reproductive organ of the plant, the gametophyte in botanical terms, which had successfully developed in the ISS. The conclusion that we can get from this series of space experiment is clear: despite the stress for plant growth and development caused by the space environment, and specifically by microgravity, plants are capable of overcoming the adverse circumstances, removing the obstacles and achieve a successful development until the adult stage, including reproduction. Therefore, the key word for future research is “adaptation mechanism(s)”. Now, a major challenge for plant space research is to know how and when the plant triggers mechanisms of adaptation in order to attenuate and even to overcome the survival problems associated with the weightless environment.

7 The Role of Light: The Seedling Growth Project.

One of the key points in the research strategy to identify the mechanisms of adaptation of plants to the microgravity environment is to determine whether, in the absence of gravity, plants may rely on other environmental cues to initiate the morphological responses necessary for their successful growth and development. These different environmental cues could act as countermeasures to counteract the adverse effects of the gravitational stress [56]. Light may act as a replacing tropistic stimulus of gravity, driving plant growth in microgravity. Novel phototropisms affecting the root were identified in space, which do not exist on ground [57]. Furthermore, light is a fundamental regulator of many physiological processes related to plant growth and development through the action of phytochromes. Specifically in relation to cell proliferation and growth, there are numerous evidences indicating that light signaling controls cell cycle and showing the activating effect of red light irradiation on the mitotic index and on the rate of ribosome production [58]. Based on these premises, the series of space experiments “Seedling Growth” (SG)

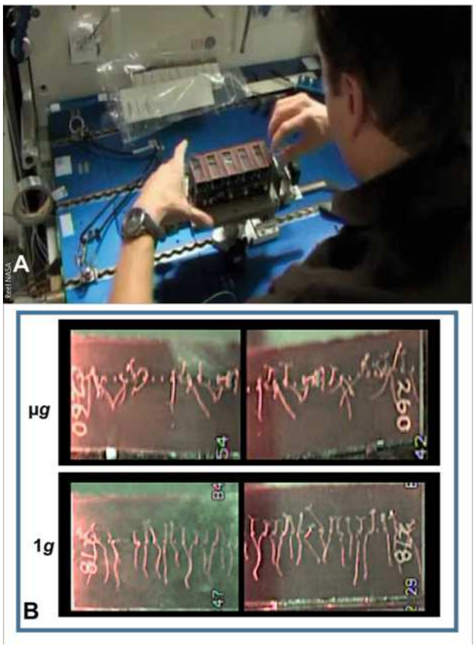

FIGURE 4. *The "Seedling Growth" experiment, performed in the International Space Station. A) An astronaut operating the culture chambers in which seedlings have grown, in order for their processing and preservation to make possible the post-flight analysis upon their return to Earth. B) Seedlings grown in space. μg: Microgravity. 1g: Control ground gravity, obtained in space by means of a centrifuge. Note the great differences in orientation of seedlings.*

were designed. The initiative of SG emerged from the confluence of the successful results of the previous spaceflight experiment "Root" in the identification of the effects of microgravity on cell growth and proliferation [28] and the long and successful series of experiments conducted by Kiss and co-workers, first in the Space Shuttle and then in ISS. In the series of "Tropi" experiments (I and II) in ISS, significant and relevant contributions were done on the changes in plant phototropism induced by microgravity in space [57, 59, 60]. Starting from these findings, the global objective of SG was to know the combined effects of light and gravity on plant development. Specifically, in addition to know how gravity and light responses influence each other in plants, and what is the combined influence of light and gravity on plant development, the SG experiments aimed to verify whether light stimulation (in particular red light) is capable of counteracting the effects of the gravitational stress on cell growth and proliferation.

SG experiments have been carried out in the period 2012-2018, and recently completed in both the spaceflight and ground segments (Figure 4). This has been one of the most complex and ambitious projects carried out in the ISS on Plant Biology up to now, as a result of the cooperation of NASA and ESA. The use of three collections of mutants, namely phototropism (phytochromes), cell growth (nucleolins) and auxin signaling, has allowed the international team to reach and publish the first results. New phototropic responses to blue light in space have been reported [61] complementing previous results of the Tropi I and II experiments. Otherwise, significant data on the positive effect of red light as a countermeasure for the stress caused by microgravity have been obtained [62]. The contribution of the nucleolin and auxin mutants to better understand how cell growth and cell proliferation are uncoupled in space is highly promising, but the analysis of these mutants by both molecular and cell biological techniques is still in progress.

8 Future Prospects

Space Biology is clearly an intellectual motivation for people, as judged for the increasing echo that the successive achievements receive in mass media and social networks. This equally refers to both sides of this discipline, namely the search for extraterrestrial life and the export of terrestrial living beings, including humans, to live and develop in extraterrestrial environments. The necessary involvement of plants in these enterprises, which includes the understanding of the mechanisms of adaptation to novel environments, unknown in the history of evolution, is one of the most important research challenges that need to be overcome. Progress in plant biology research in space has been impressive in recent years, mostly after assembly and implementation of ISS research facilities. However, the amount of pending unresolved questions is enormous and the difficulties and constraints of spaceflight research still represent a major obstacle. In order to understand the mechanisms of adaptation of plants to weightlessness, we need to discriminate with the highest precision the role played by auxin in gravity sensing and response. It is necessary to reconcile the latest results, indicating the insensitivity of the auxin transport in the root to the absence of gravity [19], with the reported alterations of the auxin flow after a change in the gravity vector. Up to now, no specific genes responsible for gravitational stress have been identified. However, most transcriptomic studies converge in that weightlessness is identified by plants as a real stress condition that triggers the general mechanisms of stress response. The fact that a significant proportion of genes that show changes in their regulation under microgravity environment correspond to "unknown function" [37] is an exciting result for further progressing in the identification of these functions. These are only two examples of the kind of the problems that need to be solved. Besides these questions of fundamental biology, it is clear that research should move from model systems to real crop species if we want to advance towards a real "space agriculture". We already have sophisticated facilities for plant culture capable of providing efficient watering, controlled air composition, low energy lighting and controls for temperature, humidity and other environmental factors. We know that the soils of Mars and of the Moon are able to support the culture of terrestrial plants [63] and that Mars gravity level is enough to be sensed by the gravitropic systems of the root [64]. These are solid bases for continuous progresses towards the achievement of real "Mars greenhouses" that today are only conceived as artist creations or scenes in movies. Finally, a highly exciting alternative use of plants in space exploration enterprises is provided by the enormous potential of the modern technologies of genetic engineering. Using genetically modified plants, we could get from them raw materials and even pharmaceutical substances, which are unable of being carried from the Earth and stored for the long periods required for space exploration. An example showing that this possibility is already coming to reality is an exciting, imaginative and innovative project (BioGalaxy) submitted by a group of Spanish students to the International iGEM Competition (`http://2018.igem.org/Team:Navarra_BG`).

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