24

Growing Plants under Generated Extra-Terrestrial Environments: Effects of Altered Gravity and Radiation

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24.1 Introduction: Plants and Space Exploration

The coming enterprises of space exploration will surely require the cultivation of plants, not only as part of Bioregenerative Life Support Systems (BLSS), but also as a source of psychological well-being for space travelers. Considering that gravity plays a unique role in the configuration of a normal plant developmental pattern, only comparable with the effect of light, weightlessness is a major stress condition that should be fully understood.

More than 50 years have passed since the first experiments performed in Space demonstrated that seed germination and plant growth can happen under conditions of altered gravity. Although with sometimes-contrasting results, data from experiments in real or simulated weightlessness indicate that microgravity itself does not prevent plant growth and reproduction; besides, the *seed-to-seed* cycle can be accomplished for several consecutive generations [1]. Indeed, during the evolution, being non-migrating organisms, plants have developed a high plasticity to adapt to changing environmental conditions. Such a high plasticity is the basis for coping with the limiting factors of extra-terrestrial environments. Morpho-functional changes due to microgravity at different plant levels have been attributed to the direct effect of microgravity on common metabolic pathways and on subcellular processes. Moreover, the interaction between microgravity and other factors, either Space-related (e.g., high levels of radiation) or distinctive of BLSS (e.g., atmospheric composition of the closed systems), can be more effective in determining growth and reproductive aberrations than the sole altered gravity [2].

Such environmental factors include quantity and quality of light, atmosphere composition of the pressurized modules, availability of water and nutrients, which may occur at suboptimal levels and would act synergistically to gravity alteration, enhancing the effects of gravitational stress. The mechanism of this synergistic effect in microgravity environments is only partially understood although a number of model systems have been successfully exposed to both real spaceflight and simulated microgravity conditions [reviewed by 3]. In fact, those effects are even more obscure under fractional gravity conditions, like the ones on Mars (0.37 g) or the Moon (0.17 g) surface. In fractional gravity conditions, some of the mechanisms that are disturbed in microgravity could still be affected (e.g., reduced graviresistance in cell walls) but others would remain unaltered (e.g., still working gravitropic responses at those gravity levels). It has been hypothesized that the two components of the gravity vector, namely direction and magnitude, could be sensed differentially in these conditions [4].

In terms of research, to generate on Earth a reduced-gravity environment is also not straightforward and requires a comparable compromise between good quality of simulation and side effects of the technology used for microgravity simulation. For instance, magnetic levitation of samples provides a very stable partial-gravity environment, but adds a new layer of complexity due to the presence of high magnetic fields in the equation. Research on plant gravitropic responses has also profited by experiments in hypergravity conditions simulated through centrifuges [5–7]. It should be reminded here the usefulness of hypergravity research in other biological systems to learn about diseases as osteoporosis or sport training under overloading environments.

The accurate identification of stress factors, and the strategy followed by plants in their adaptation to an extra-terrestrial environment, will be necessary for achieving a successful cultivation of plants on board of spaceships and, in general, outside the Earth environment, which is essential to make manned space exploration possible.

In this section, we report an overview of the current knowledge about plant's responses to altered gravity from molecular to the whole organism level. In the light of long-lasting experimentation in space, or in simulated space conditions, the influence of ionizing radiation and possible interferences of other environmental factors acting at suboptimal levels in ecologically closed systems are also considered as additional stressors for the achievement of efficient plant growth.

24.2 Cellular and Molecular Aspects of the Gravity Perception and Response in Real and Simulated Microgravity

24.2.1 Gravity Perception in Plant Roots: Gravitropism

Among environmental factors influencing plant growth, development, survival, and evolution, gravity is characterized by its permanent and constant presence on Earth. It is long-time known that plants are sensitive to the presence of the gravity vector which drives plant growth direction by a phenomenon called gravitropism. The process is triggered by the displacement of amyloplasts (statoliths) in the columella root cap cells [8].

The mechanism of plant root gravitropism involves, first, the transformation of the mechanical signal into a biochemical signal, and then, the transduction of this signal from the site of sensing to the site of response; this process is mediated by the phytohormone auxin and its polar transport throughout the root length [9, 10] (Figure 24.1). This mechanism has been

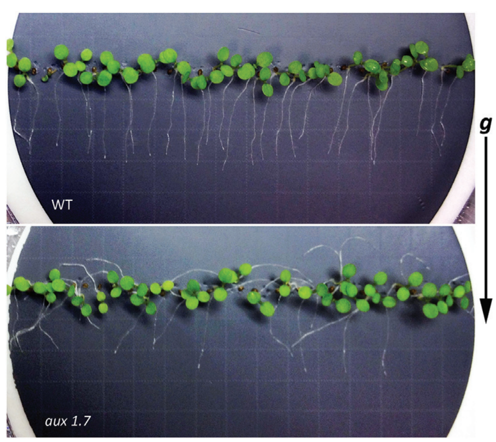

Figure 24.1 Seedlings of *Arabidopsis thaliana*. The upper image shows the wild type and the lower image the agravitropic aux 1.7 mutant. Seedlings of the wild type show conspicuous gravitropic behavior, with the roots aligned in the direction of the gravity vector; however, aux 1.7 mutant seedlings show evident alterations of gravitropism with roots growing in random directions.

mainly elucidated by experiments consisting of changing the orientation of growing seedlings. It was shown that a short time after seedling re-orientation, the root changes the direction of its growth according to the gravity vector. Root bending associated with the change in growth direction is caused by the alteration of the auxin polar transport, leading to the lateral re-distribution of auxin in the distal regions of the root, which eventually results in a different rate of elongation of cells located at both sides of the root in a specific zone (elongation zone) [11]. This morphological event is accompanied by a substantial reorganization of gene expression [12].

An important source of information on the mechanisms of gravitropism comes from studies performed in the absence of gravity. On Earth, real weightlessness is obtained by free fall; however, values of the gravity vector near zero (lower than 10^{-6} g) occur in long-duration missions, in spaceships orbiting the Earth, such as the International Space Station (ISS), or, previously, the Space Shuttle.

In early experiments in the Spacelab, it was shown that lentil root cell statoliths did not distribute at random, but they accumulated in the proximal region of columella cells [13]. In fact, statoliths are attached to actin filaments by means of myosin, and this greatly affects their displacement within the cell [14]. Otherwise, the use of starchless *Arabidopsis* mutants in spaceflight experiments provided support to the starch-statolith model for gravity sensing [15].

Space experiments have provided support to the role of auxin polar transport as a fundamental mediator of the transduction of the gravity signal. Seedlings grown in space showed alterations in the auxin polar transport [16], and genes related to this process have been found to be differentially regulated in spaceflight- or parabolic-flight-grown samples [17–19].

The intrinsic constraints of space experimentation, mostly the limitations of the general access to spaceflights, induced the development of ground-based devices capable of a reliable simulation of microgravity conditions. For this purpose, devices capable of counteracting the perception of the Earth gravity vector by plants, called clinostats, were designed and constructed. The first classical clinostats were introduced by Sachs in 1879 and allowed to work with simulated microgravity on Earth [reviewed by 3]. It is important to emphasize, however, that these devices, including the most modern ones, such as the random positioning machine (RPM), do not suppress the gravity vector, but they only act at the level of the mechanism by which living beings perceive it. In addition, recent technologies such as diamagnetic levitation can be used to produce a weightlessness environment for plants. This technology takes advance of the major presence of diamagnetic materials in organisms (mostly water): by producing a high gradient of magnetic field into the small volume of the sample container in the magnet bore (a tube of around 5 cc), a strong levitation force is produced, which is capable of counteracting gravity. Although this technology has the advance of producing a continuous (not averaged) effect, even detectable at the molecular level, it has the disadvantage of adding side effects, mostly the magnetic field itself, into the equation. On the other hand, it allows performing several partial *g* experiments (in the gravity range between 0 and 2 g) at the same time and in the same environment [3].

Ground-based facilities for microgravity simulation have demonstrated their usefulness in gravity-related research. In some cases, differences between the statolith position in clinostats and in space have been reported [13], but, in general, results are largely comparable [20].

24.2.2 Effects on Cell Growth and Proliferation

Altered gravity produces changes in plant development, which are closely associated with modifications in the activities of meristems, specifically cell growth and cell proliferation/cell cycle. All adult plants contain meristematic tissues, composed by populations of undifferentiated, highly proliferating cells, capable of forming any specialized tissue at any time in the life span of the plant. Indeed, plant development greatly depends on the balance between cell proliferation and cell differentiation in the meristems; these phenomena are controlled, in turn, by the auxin [21]. Results obtained in space experiments showed altered cell proliferation rate in both lentil [22] and *Arabidopsis* [23]. Under microgravity conditions, cell proliferation and cell growth appear uncoupled, losing their coordinated progress which is called “meristematic competence” and is the main feature of these cells under normal ground gravity conditions [9, 23, 24].

It should be pointed out that spaceflight not only causes gravitational stress, but it also involves additional environmental modifications with respect to the Earth conditions, such as the confinement, the lack of gas convection, and the cosmic radiation, which may be sensed by plants, causing additional stresses [25]. For this reason, the results obtained in real microgravity have to be sustained by studies carried out on ground, using the available facilities for microgravity simulation [3] in combination with some of the stressors that could be present in extraterrestrial habitats.

24.2.3 Effects of Gravity Alteration on Gene Expression

A few studies on gene expression have been carried out in real or simulated microgravity, by performing an overall transcriptomic analysis of seedlings or solid cell cultures (calluses), to search for gravity-regulated genes. Experiments included reorientation of seedlings [12], cell cultures grown in ground-based facilities for microgravity simulation [26, 27], and growth of seedlings during parabolic flights [19] or in spaceflight conditions [17, 18]. These studies have revealed a complex response of plants to gravity alteration, including changes in the expression of genes involved in general responses to other stresses, such as drought, cold, light, and biotic stimulation by pathogens. Heat shock-related genes, cell wall remodeling, and cell expansion genes, as well as genes involved in oxidative burst and plant defense, are outstanding examples of transcriptomic alterations shared by different kinds of plant stresses, including the gravitational one, and commonly reported in different experiments. However, in most studies, the existence of a specific response to gravity alteration was suggested or postulated. Specific alterations affect genes involved in the gravitropic response, such as those related to hormone signaling, particularly auxin [17–19], and also a number of genes of unknown function. Furthermore, there is some specificity in the process of adaptation to spaceflight environment by different organs of the plant, which engages different genes, even though the general strategy of response is shared in all cases [18]. An additional specific feature of gravity, as a permanent component of the environment, is that it is capable of altering the perception and response of living organisms to other environmental factors [26, 28].

24.3 Morpho-Functional Aspects of the Plant Response to Real and Simulated Microgravity Environments

24.3.1 From Cell Metabolism to Organogenesis

Organogenesis is a complex phenomenon requiring a precise coordination of cell proliferation, enlargement, and differentiation, which are regulated by gene expression and signaling biologically active molecules. The mechanisms linking cell division and the whole plant development have not been completely understood yet [29]. As reported above, microgravity surely affects cell proliferation which is the first step for plant growth; in addition, cell enlargement and differentiation can be modified in altered gravity conditions. Most of the knowledge about the effect of microgravity on morphogenesis in higher plants derives from experiments where seedlings, being the smallest yet complete form of a plant, have been used as models due to volume and time constraints. The whole plant life cycle has been investigated only in a few plant species characterized by small size, such as *Arabidopsis* spp. and *Brassica* spp. [2]. Indeed, vegetal systems characterized by reduced size better match with volume constraints in the experimental facilities in space and do not encounter bending problems during clinorotation. The interest in studying seedlings also derives from their high nutritional value which makes them suitable candidates to reintegrate the crew's diet with fresh food [30].

Morpho-anatomical changes detected in plants growing under real or simulated microgravity have been often considered primarily as the consequence of altered cytoskeleton organization, increased production of reactive oxygen species (ROS), and modifications in starch and phenylpropanoid metabolism. Cortical microtubule organization has been demonstrated to be affected by reduced or increased gravity levels in both gravisensing cells and in cells not specialized to gravity perception in isolated protoplasts, root, and other organs [31–33]. The alteration of spatial organization of cytoskeleton microtubules has influence on cell growth because it not only regulates the cytoplasm expansion, but also affects cellulose microfibrils orientation during cell wall development, which in turn controls protoplast expansion [34, 35]. Increased or decreased cell enlargement, sometimes ascribed to the accumulation of ethylene in the experiment containers during spaceflight, have been related to changes in seedling size [36]. The size of roots or hypocotyls was found to increase, decrease, or remain unchanged in seedlings of various species exposed to clinorotation or real microgravity (see, for example, [37, 38]). Relatively few studies have focused on leaf development in space also with contrasting results. Stutte et al. [39] showed that the minimal space-induced modifications in leaf structure (e.g., reduced thickness, more dense mesophyll, and altered chloroplast morphology accompanied by unchanged contents in starch, soluble sugars, and lignin) of *Triticum aestivum* did not cause any physiological changes. These authors suggested that severe alterations in the (ultra)structure of tissues and organelles, found in early spaceflight experiments, were caused by an inadequate environmental control in the experimental chambers.

24.3.2 Indirect Effects of Altered Gravity to Photosynthesis

The maintenance of high photosynthetic rate is needed to optimize O_2 production and CO_2 removal in BLSS. Experiments conducted on dwarf wheat on the ISS showed that microgravity does not alter the development of the photosynthetic apparatus and the efficiency of photosynthesis [39]. While no changes in chloroplasts membranes were found in flown *Arabidopsis thaliana*, changes in the thylakoid structure occurred in *T. aestivum* and *B. rapa* (reviewed in [31]). Moreover, a decrease in the electron transport rate of photosystem I (PSI), measured in *B. rapa* suggested the major susceptibility of the PSI in comparison with PSII. It has been recently highlighted that some of the structural and physiological changes of the photosynthetic apparatus in space should not be considered as the result of a direct effect of microgravity, but as the consequence of starch accumulation due to the delayed long-distance transport of photosynthetic metabolites [31, 40].

24.3.3 Constraints in the Achievement of the Seed-to-Seed Cycle in Altered Gravity

The reproductive cycle is characterized by a succession of highly specific phases where mitosis and meiosis, as well as cell enlargement, follow one another in a few specialized cells. Whatever the factor affecting cell cycles and cell growth, it can also affect one or more reproductive phases, thus ultimately endangering seed set.

There is a common agreement that the reproductive failure claimed in the early experiments performed in space was triggered by inefficient environmental control in the growth chambers resulting in too high humidity, accumulation of ethylene, and insufficient carbon supply (reviewed in [2]). Problems in ventilation have been considered responsible for the interruption of the plant life cycle in different phases, such as the transition from vegetative to reproductive stage, microsporogenesis, female gametophyte formation, anther dehiscence, pollination, and embryo development. Recent advances in space-related technologies allowed better control of growth chambers, thus facilitating the completion of the *seed-to-seed* cycle in various model species [41, 42]. However, there is evidence that the quality of seeds produced in microgravity is not optimal: seeds of *B. rapa* formed in microgravity were characterized by smaller size and different composition of the reserves compared to controls, probably because microgravity affects the microenvironment inside the silique [42]. Finally, it cannot be disregarded that some reproductive phases are strictly controlled by the coordinate activity of intracellular components, particularly cytoskeleton, which is affected by microgravity. For example, pollen tube development in simulated microgravity is subjected to alterations which could reduce its capacity of fertilization [43]. To conclude, alterations in different reproductive phases, due to direct or indirect effects of microgravity, can either completely interrupt the reproductive cycle or determine progressive lowering of the reproductive success.

24.4 Plant Response to Real or Ground-Generated Ionizing Radiation

24.4.1 Variability of Plant Response to Ionizing Radiation

Space radiation represents a major barrier to human exploration of the solar system because of the biological effects of high-energy and charge (HZE) nuclei [44]. The degree of damage is associated with different radiation types. High-linear energy transfer (LET)-ionizing radiations (e.g., protons and heavy ions) have lower penetrating capability but are more dangerous than low-LET ones (e.g. X- and gamma-rays) for both plant and mammalian cells [45–47].

The current knowledge about the biological effects of radiation on photosynthetic organisms comes from experiments performed in long- and short-duration space missions. Additional sources of information derive from ground-based experiments conducted by means of accelerators testing different ions and energy ranges on diverse species. Despite the high variability in the results, which often makes data hardly comparable, a common view is that ionizing radiation can have stimulatory effects at very low doses, harmful consequences at middle levels, and detrimental outcomes at high doses on plant development [46]. The severity of the effects is dependent upon several factors including the species, cultivar, phenological phase, and genome organization [48].

24.4.2 Effects of Ionizing Radiation at Genetic, Structural, and Physiological Levels

Radiation-induced alterations are generally ascribed to the interaction of the radiation itself with atoms and molecules, which causes the production of ROS [49].

At genomic level, the structural and functional changes in the DNA are responsible for most of the damage expressed after the exposure to ionizing radiations. The nature of DNA modifications is variable and includes single-base alterations, base substitutions, base deletions, chromosomal aberrations, and epigenetic modifications. Generally, chronically irradiated samples show a higher genomic instability compared to acutely exposed samples [50].

Radiation-induced morpho-functional changes can be either the phenotypical expression of DNA aberrations or the consequence of the structural damage of tissues. The main alterations include reduced germination, lethality, loss of apical dominance, induction of dwarf growth, altered leaf anatomy, sterility, and accelerated senescence [46, 51]. The degradation of cell wall materials determines tissue softening, due to the dissolution of middle lamellae, and the increase in seed germination, ascribed to increased water absorption due to augmented porosity of seed teguments [52, 53].

Among processes regulating plant life, photosynthesis is one of the most sensitive to radiation. Several components of the photosynthetic machinery may be altered: light-harvesting complexes, electron transport carriers, and enzymes of the carbon reduction cycle [51, 54]. The severity of damage depends on dose; the loss of PSII functionality, and the generation of ROS throughout the cell are observed at very high doses.

The exposure to low doses of ionizing radiation may induce radioresistance [55]. Plants are very radioresistant compared to animals. At cellular level, radioresistance is achieved through the occurrence of mechanical and chemical barriers (e.g., specialized and thickened cell walls, cuticle, pubescence, and phenolic compounds), as well as by increasing the level of ploidy [45, 56, 57]. The chronic exposure to low doses of ionizing radiation also leads to significant differences in the expression of radical scavenging enzymes and DNA-repair genes as well as to an increase in the activity of several antioxidant enzymes in plant tissues [55].

24.5 Conclusions—Living in a BLSS in Space: An Attainable Challenge

More than 50 years of experiments with higher plants in space suggest that almost all developmental and reproductive phases can be successfully overcome, ultimately leading to accomplish the *seed-to-seed* cycle for successive generations. However, possible perturbations can happen at various levels, from molecular to organism, thus lowering the efficiency of growth and reproduction processes. Such perturbations can be ascribed to the interaction between space factors and other environmental factors not acting at optimal levels.

The incessant development of new agro-technologies is promising for the development of BLSS with adequate environmental control to reduce the effect of multiple stressors on plant growth. Ground-based research aiming to the development of BLSS heads for soilless systems to minimize other potential stresses during plant cultivation [58]. However, in space, it is necessary to minimize the use of resources (e.g., lighting, electric power, water, and nutrients consumption). Consequently, a compromise needs to be reached to minimize external supply of resources, still without overcoming the threshold over which suboptimal levels of environmental factors may worsen the effects of microgravity and other space factors on plants. While new agrotechnologies progress, the ground-based research with facilities simulating weightlessness, ionizing radiation, and confined environments also needs to advance. Such studies are necessary to elucidate the plant's sensitivity to space factors in the sight of wider space experimentation.

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