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ORIGINAL ARTICLE

Higher Plants in Space: Microgravity Perception, Response, and Adaptation

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Abstract Microgravity is a major abiotic stress in space. Its effects on plants may depend on the duration of exposure. We focused on two different phases of microgravity responses in space. When higher plants are exposed to short-term (seconds to hours) microgravity, such as on board parabolic flights and sounding rockets, their cells usually exhibit abiotic stress responses. For example, Ca^{2+}-, lipid-, and pH-signaling are rapidly enhanced, then the production of reactive oxygen species and other radicals increase dramatically along with changes in metabolism and auxin signaling. Under long-term (days to months) microgravity exposure, plants acclimatize to the stress by changing their metabolism and oxidative response and by enhancing other tropic responses. We conclude by suggesting that a systematic analysis of regulatory networks at the molecular level of higher plants is needed to understand the molecular signals in the distinct phases of the microgravity response and adaptation.

Keywords Microgravity · Higher plant · Response · Adaptation

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Introduction

Plant microgravity science has been one of the most active fields in space biology since the first satellite was launched in 1957. Studies on higher plants in space have two main goals: bioregenerative life support systems for long-term missions and fundamental plant biology research. Long-term human space exploration missions require a life support system capable of regenerating all the essentials for astronauts' survival. Plants are considered a key factor in such a bioregenerative system, because photosynthesis can provide food, such as carbohydrates, proteins, lipid oils, and vitamins, as well as the oxygen that is vital to maintaining a balance between the partial pressures of oxygen and carbon dioxide. Studies on plants in bioregenerative life support systems have been extensively reviewed (Salisbury and Bugbee 1988; Kliss et al. 1994; Tako et al. 2010; Wheeler 2010; Paul et al. 2013a). This review will focus on short-term and long-term space experiments using higher plants, with an emphasis on their perception of, responses to, and adaptation to microgravity under spaceflight conditions (Fig. 1).

The ability of plant organs to use gravity to guide growth is called gravitropism; gravitropism maximizes the uptake of water and nutrients from the soil by roots and solar energy capture by leaves on the ground. Many studies have demonstrated that plant growth is severely impaired in space (Briarty and Maher 2004; De Micco et al. 2011; Paul et al. 2013b). Plants grown in microgravity (i.e., on spaceflights) or in simulated microgravity (i.e., on clinostats) exhibit spontaneous curvatures or changes in growth direction, called automorphosis (Hoson et al. 1995; Driss-Ecole et al. 2008) or automorphogenesis (Stanković et al. 1998). Recent advances in plant genomics and proteomics and microgravity experiments on spaceflights, which allow real

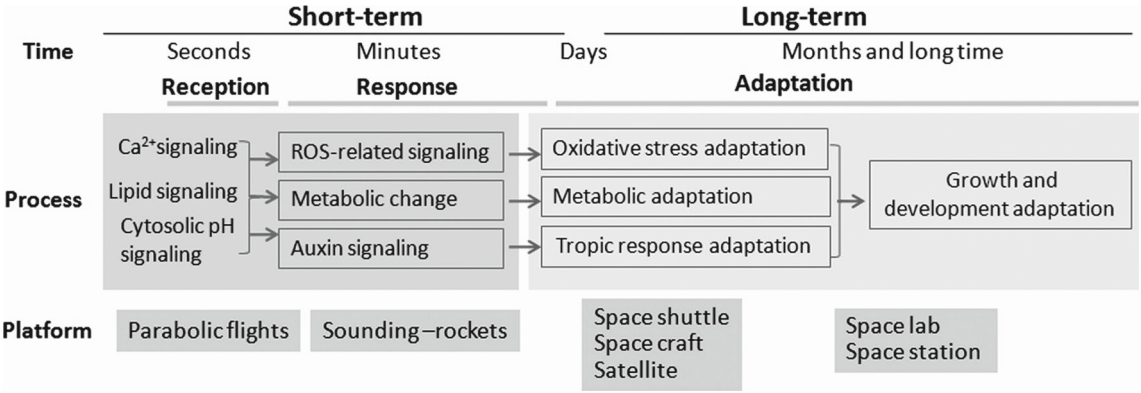

Fig. 1 Schematic diagram of short- and long-term responses of plant cells toward microgravity

changes in the orientation and/or intensity of the gravity vector, allow the mechanisms of perception, response, and adaptation of plants to microgravity signals to be addressed. The action of microgravity has been hypothesized to depend on the duration of exposure (Claassen and Spooner 1994; Matía et al. 2009). Upon exposure to short-term (seconds to hours) microgravity, plant cells initially exhibit abiotic stress responses, such as dramatically increasing the production of reactive oxygen species (ROS) and other radicals, while in long-term (days to months) microgravity, plants change metabolically and adapt to oxidative stress (Tables 1 and 2). However, key questions remain. How does microgravity affect plant growth and development? Do particular molecular signals exist? And, if so, are they specific to the distinct phases of microgravity response and adaptation?

Drop towers, sounding rockets, and aircrafts during parabolic flight can be used to offer a short-term microgravity. Although the duration of these platforms is very short (seconds to minutes), it is enough to cause signaling changes in calcium, lipids, and cytosolic pH that alter ROS signals, the redistribution of auxin, and changes in metabolic activity leading to an adaptive plant response. The opportunities for long-term space experiments are very scarce. Therefore, ground-based facilities, such as clinostats, have been developed to simulate microgravity, but the influence of gravity will never fully be neutralized on the ground (Briegleb 1992).

Satellites, spacecraft, and space shuttles can offer relatively long-term days to months) microgravity. Many of the insights into fundamental plant biology in space were enabled by these types of space platforms. For example, the effects of microgravity on overall plant metabolism, growth, development, and reproduction were determined (Zheng et al. 2008; De Micco et al. 2014). The genomic and proteomic responses of plants to space flight were also analyzed (Table 1 and 2). The upmass and capsule volume are still limiting factors for the study of long-term plant responses to space environments.

Reception and Response

Strong evidence now shows that plants can perceive gravity on the time scale of a second. For example, the sedimentation of statoliths (amyloplasts) in gravity-sensing columella cells of *Arabidopsis* roots occurred in less than 1 s (Hejnowicz et al. 1998; Perbal et al. 2002; Perbal and Driss-Ecole 2003), and the redistribution of auxin and root curvature growth could be observed within 10 s (Leitz et al. 2009). However, the underlying molecular mechanisms of these responses in plant cells remains unknown. Numerous signaling pathways, such as Ca^{2+}, lipid, and cytosolic pH, have been implicated in short-term microgravity-induced signal transduction (Table 1).

Calcium Signaling

Environmental changes are signaled through a transient increase in the intracellular Ca^{2+} concentration, which is also thought to be an important second messenger for sensing and responding to gravity (reviewed in Plieth 2005, Toyota et al. 2008). The plasma membrane is considered the primary site for sensing microgravity, which causes an influx of Ca^{2+}. The movement of Ca^{2+} in the wall might regulate extension growth and that free intracellular Ca^{2+} might mediate signaling in statocytes in response to an altered gravity vector. However, the mechanistic understanding of the role of Ca^{2+} in response to microgravity still lack (Leitz et al. 2009) Recently, the responses of cytosolic calcium, hydrogen peroxide H_2O_2), and related gene and protein expressions in *Arabidopsis* cell cultures on parabolic flights has been studied (Hausmann et al. 2014). Ca^{2+}-dependent genes, such as members of the Ca^{2+}–binding family and Ca^{2+}-dependent protein kinases, exhibited increased expression at the end of the microgravity phase of the parabola (Hausmann et al. 2014). A close interrelationship between Ca^{2+} and ROS signaling was also reported (Wong et al. 2007; Takeda et al. 2008). For example,

Table 1 Current publications focusing on genomics and proteomics of plants under microgravity according to PubMed

Species	Treatments	Results	References
Short-term microgravity			
Arabidopsis roots (WT, *pin2*, *pin3*)	Parabolic flights	Expression of genes involved in lipid metabolism, stress factors and light categories significantly changed in response to transient microgravity phase.	Aubry-Hivet et al. 2014
Arabidopsis thaliana callus	Parabolic flights	396 transcripts were specifically up-, while 485 genes were down-regulated. Up-regulation was dominated by Ca^{2+} and ROS^{-} related gene products.	Hausmann et al. 2014
Arabidopsis thaliana plants	Parabolic flights	Altered expression genes in response to the earliest (20 parabolas) were associated with signal transduction, while those in response to the later (40 parabolas) were associated with biotic and abiotic stress.	Paul et al. 2011
Arabidopsis thaliana callus	Sounding-rocket (10 min), RPM, hypergravity (8g)	Nine genes (mainly components of signaling chains) were differentially expressed in response to microgravity.	Martzivanon et al. 2006
Etiolated sunflower	Sounding –rockets, Fast clinorotion	Inositol 1,4,5-trisphosphate, Ran exhibited altered expression.	Kriegs et al. 2006
Long-term microgravity			
Arabidopsis thaliana	ISS	480 gene altered expression at least 1.9-fold associated with cell architecture and growth, hormone signaling.	Paul et al. 2013b
Arabidopsis thaliana	ISS	230 genes (1g in space *vs.* 1g on ground), 280 genes (microgravity *vs.* 1g on ground) and 27 genes (microgravity *vs.* 1g in space) differentially regulated by at least two-fold. These altered expression genes involved in regulating cell polarity, cell-wall development, oxygen status, and cell defense or stress.	Cornell et al., 2013
Arabidopsis (WT, *tua6* mutant)	ISS	Cortical MTs generally play an important role in plant resistance to the gravity force.	Hoson et al., 2014
Zebrawood seedlings	ISS	Large and small heat shock proteins, 14-3-3-like protein, polybiquitin proteins and peroxidase exhibited altered expression under microgravity.	Ingis et al., 2014
Arabidopsis thaliana seedlings	ISS (10d)	227 membrane proteins displayed no abundance differences (μg *vs.* 1g in space), while their abundance significantly different between 1g in space *vs.* 1g on ground. 176 were up- in space involved in protein synthesis, degradation, transport, lipid metabolism, or ribosomal protein, 51-down regulated involve in membranes, aquaporins and chloroplastic proteins.	Mazars et al., 2014
Dwarf wheat	ISS (21d)	No difference in gene expression was detected, suggesting that the space flight environment had minimal impact on wheat metabolism.	Stutte et al., 2006
Arabidopsis thaliana callus	STS-131, hyper-g (3g or 16g), 2D clinostat, Parabolic flights	Hsp17.6A and Hsp101, HSP genes were induced in response to sustained clinorotation, transient microgravity intervals in parabolic flight, but various hypergravity conditions failed to induce HSP genes.	Zupanska et al., 2013

Table 1 (continued)

Species	Treatments	Results	References
Arabidopsis thaliana plant (*Pro Adh::GUS*)	STS-93, 5-day	182 genes were differentially expressed in response to the spaceflight mission by more than 4-fold. Genes related to heat shock were dramatically induced. The *Adh/GUS* reporter gene was activated in roots, but was not in shoot apex during the flight.	Paul et al., 2005
Cucumber seedlings peg	STS-95, Clinostat	4-fold reduction in the expression of CsExp3 under clinostat vs. those grown at 1g, but no differences in the expression of CsExp3 or CsExp4 under microgravity in space vs. those under 1g in space.	Link et al., 2001
Tobacco cell cultures	Micro-g	A much lower activity of caffeic acid O-methyltransferase (lingnin biosynthesis) under microgravity. Gene expression under microgravity was not different from 1g control in space.	Sato et al., 1999
Euglena gracilis	Chinese spacecraft SZ-8	Altered expression of several genes involved in signal transduction, oxidative stress defense, cell cycle regulated and heat shock response.	Nasir et al., 2014
Arabidopsis thaliana callus	Chinese spacecraft SZ-8 (14d)	45 proteins showed differentially expressed in response to microgravity vs. those under 1g in space. The functions of these proteins were involved in general stress responses, carbohydrate metabolism, protein synthesis/degradation, intracellular trafficking/transportation, signaling, and cell wall biosynthesis.	Zhang et al., 2015
Arabidopsis thaliana callus	Chinese spacecraft SZ-8 (5d)	The genes, which altered expression under spaceflight condition, involved in the plastid-associated translation machinery, mitochondrial electron transport, and energy production.	Fengler et al., 2015

seconds of exposure to microgravity could increase both cytosolic Ca^{2+} and H_2O_2 levels in parallel (Hausmann et al. 2014). Ca^{2+} influx caused by microgravity can activate a Ca^{2+}-dependent protein kinase (CDPK), which phosphorylates the N-terminal region of the H_2O_2-producing NADPH oxidase, thus increasing H_2O_2 production. The latter led to the up-regulation of H_2O_2 scavengers such as ascorbate peroxidases (APX4, APX6), glutathione peroxidase, catalases (CAT1), and superoxide dismutase. H_2O_2 production and degradation will consequently reach a new balance in long-term microgravity.

Lipid Signaling

Membrane lipids can serve as signaling molecules in addition to their structural function because of their specific distribution in cellular compartments (Toker 2002). Most of these molecules, such as phosphatidylinositol (PtdIns) and its phosphorylated derivatives, the water-soluble inositol (1,4,5), trisphoshate (InsP3), sterols, and sphingolipids, are involved in vesicle trafficking, which is essential for the auxin transport facilitators PINs recirculation during gravitropic responses (Smith et al. 2013; Boutté and Grebe 2009). InsP3 is involved in releasing intracellular calcium after altered gravity stimuli, and its signal peaks appear at both the sensing site and elongation zone of roots (Krinke et al. 2007; Im et al. 2010; Gillaspy 2011; Perera et al. 1999, 2001). Although the downstream receptor of InsP3 is still unknown, it participates in regulating the redistribution of auxin in response to altered gravity. For example, when InsP3 was hydrolyzed or its synthesis blocked by chemical inhibitors like U73122, the establishment of an asymmetric auxin distribution in roots was delayed, and gravitropic response was also attenuated (Perera et al. 2001; Andreeva et al. 2010; Salinas-Mondragon et al. 2010).

Cytosolic pH Signaling

The pH in apoplasts of central columella cells of roots increased from 7.2 to 7.6 during statolith sedimentation (Fasano et al. 2001). This alkalinization may be an additional result of calcium ion channel activation, which usually activates a plasma membrane H^+/OH^-conductance (Boonsirichai et al. 2006; Monshausen et al. 2011; Sato et al. 2014). However, this alkalinization is absent in starchless *pgm* mutants (Fasano et al. 2001). Transcriptomic data obtained in microgravity showed that many genes are closely related to calcium processes and cytosolic pH signaling (Paul et al. 2011, 2012). The capacity to regulate cytoplasmic pH has been suggested to be a crucial factor in determining cell survival under microgravity.

Table 2 Current publications focusing on the response of genomics and proteomics of plants under simulated microgravity and/or hypergraivity conditions according to PubMed

Species	Treatments	Results	References
Arabidopsis thaliana callus	Magnetic field, 3-D clinorotation, 2g hypergravity	Differential expression genes involved in structural, abiotic stress, secondary metabolism	Manzano et al., 2012
Arabidopsis thaliana seedlings	3-D clinorotation	For the 1h of 3D clinorotation, about 400 genes exhibited differential expression, with 302 up-regulated and 110 down-regulated genes, encoding for ROS-related enzymes, transporters, transcription and metabolism. 325 up-regulated and 177 down-regulated gene were scored for the long-term (6 days) exposure to simulated microgravity. Genes for antioxidant and enzyme regulators specifically expressed in the seedlings exposed to long-term 3-D clinorotation.	Soh et al., 2011
Arabidopsis thaliana seedlings	3-D clinorotation	Differential expression gene 177, involved in general metabolism, biogenesis of cellular components, cellular transports and transport facilitation, cell rescue and defense response.	Kittang et al., 2004
Arabidopsis thaliana callus	Hypergravity, simulated microgravity (2-D/3-D clinorotation, magnetic levitation)	Changes in gravity induced stress-related signaling, exposure in the 3-D clinorotation induced changes in gene expression which resemble those of magnetic levitation. 2-D clinorotation resulted in responses similar to those caused by hypergravity.	Babbick et al., 2007
Arabidopsis and corn seedlings	3-D clinorotation, hypergravity	Selective and significant differences in gene expression were observed in simulated microgravity and hypergravity treated plants. Calcium/calmodulin-mediated gravitropic response in plants was discussed.	Poovaiah et al., 2002
Arabidopsis the floral stem and rosette leaf	Hypergravity (300g)	The expression of XTH genes changes in response to hypergravity of 300g	Zenko et al., 2003
Etiolated pea seedlings	Hypergravity(3-14g)	Expression of Hsp70 and Hsp 90 increased under hypergravity and a tendency towards recovery of the normal content during re-adptation	Kozeko and Kordyum, 2009
Etiolated pea seedlings	3-D clinorotation	Expression of PsPINs and PsAux1 is under the control of gravistimulation and relevant to automorphesis	Hoshino et al., 2004
Solamun lycopersicum	3-D clinorotation	MiRNAs: six of seven miRNA up-regulated, which are involved stress response, transcription regulation, signal transduction	Xu et al., 2013
Arabidopsis thaliana callus	Magnetically induced hyper- and microgravity	Combination of gravitation alteration and magnetic field exposure produced synergistic effect on the proteome of plants. 19 abiotic stress and secondary metabolism proteins were analyzed.	Herranz et al., 2013

Table 2 (continued)

Species	Treatments	Results	References
Arabidopsis thaliana root tips (WT and *pin2*)	2-D clinostat rotation; hypergravity (7*g*)	25 proteins, whose expression level/pI was altered by clinorotation and/or hypergravity force, were identified in Arabidopsis WT and/or *pin2* mutant roots.	Tan et al., 2011
Arabidopsis thaliana pin2	2D clinorotation, Hyper-g, 3-D clinorotation	Phosphorylation of stress-related proteins under centrifugation and 2-D clinorotation, but this did not detect under 3-D clinorotation.	Barjaktarović et al., 2009
Arabidopsis thaliana callus	Hyper-g, 3-D clinorotation	More changes in the degree of phosphorylation occur under 3-D clinorotation treatment in comparison with those under hypergravity.	Barjaktarović et al., 2007
Arabidopsis thaliana callus	2-D clinostat rotation	18 proteins involved in general stress response, metabolic pathways, gene activation/transcription, protein synthesis and cell wall biosynthesis	Wang et al., 2006

Microgravity modifies the dynamics of fluids and small particles, thus altering the diffusion of gases (e.g., carbon dioxide and oxygen) and the uptake of water and nutrients (Wolff et al. 2014). Plant cells grown in such an environment might exhibit hypoxia. Oxygen-deficit stress induces cytoplasmic acidification. To prevent acidosis, plant cells grown under microgravity can enhance the activity of fermentative pathway (Paul et al. 2001) and probably accumulate amino acids, such as alanine, to regulate the pH within the anoxic cells. The lack of high-resolution instrumentation is a major obstacle to studying the effects of microgravity on intra- and extracellular pH (Ruyters and Braun 2014). The use of novel fluorescent pH reporter proteins, like *Ptilosarcus gurneyi* green fluorescent protein (Pt-GFP), and fluorescent biosensor technology enables more accurate measurement of intracellular pH (Monshausen et al. 2011; Gjetting et al. 2012; 2013; Geilfus et al. 2014) . Fluorescence microscopy and confocal live-cell imaging have begun to be used in space experiments (Ruyters and Braun 2014) and may provide more details about changes in intra- and extracellular pH in space.

Auxin Reflux and Signaling

Asymmetric application of H_2O_2 promoted gravitropism in maize roots, indicating the involvement of the auxin signaling pathway (Joo et al. 2001). Recently, comparisons of global differential gene expression in *Arabidopsis* wild-type (WT) and mutant (i.e.,*pin*2 and *pin3*) seedlings grown under parabolic flight conditions were performed using DNA microarrays,the results demonstrated that the regulation of auxin responsive genes in transient microgravity is PIN3-dependent. PIN2-mediated auxin responses are located downstream of the primary transient microgravity response (Aubry-Hivet et al. 2014). These data partially corroborate previous proteomic analysis of *Arabidopsis* WT and *pin2* roots under clinorotation and hypergravity conditions (Tan et al. 2011). In addition, three-dimensional clinorotation remarkably increased the expressions of *PsPIN1* and *PsAUX1* in pea epicotyls (Hoshino et al. 2004). Notably, these proteins are regulated by light as well as gravity (Ruyters and Braun 2014; Vandenbrink et al. 2014). Only a microgravity environment could distinguish the two stimuli (Vandenbrink et al. 2014). Studies have been carried out in microgravity recently on the International Space Station (ISS) (Vandenbrink et al. 2014). The impacts and cross-talk of light and gravity, nevertheless, in particular on indole-3-acetic acid transport, require further evaluation.

Metabolic Changes

Rapid metabolic responses in higher plants were also observed in short-term hypergravity and microgravity.

For example, transient microgravity enhanced carbohydrate metabolism in WT and *pin2* but not in *pin3* roots (Aubry-Hivet et al. 2014), indicating an increased demand for energy during the response. Increased phosphorylation of triosephosphate isomerase (glycolysis), pyruvate dehydrogenase (glycolysis), and citrate synthase (citrate cycle) was detected in *Arabidopsis* callus cells upon exposure to microgravity for 20 s (Hausmann et al. 2014). Thus, short-term microgravity stimulation is sufficient to alter the transcript levels of many genes, mainly Ca^{2+}- and ROS-related ones.

Adaptation

The long-term and short-term effects of microgravity on plant growth and development appear to be different. For example, a number of metabolic products, ions, and signal molecules accumulated during short-term microgravity, but their levels did not continue to increase during long-term microgravity. Long-term adaptation to microgravity is essential to plant growth and proper development in spaceflight conditions. Plants show a strong capacity to withstand long-term spaceflight conditions via metabolic changes and adaptation to oxidative stress (Table 1). Primary and secondary metabolic changes in plants on U.S. space shuttles and the ISS have been recently reviewed (Paul et al. 2013a); three main strategies are usually used by plants: altered metabolism, adaptation to oxidative stress, and enhancement of other tropic responses.

Metabolic Adaptation

Characterizing metabolic changes has been generally considered among the most direct approaches for studying physiological responses of plant cells to microgravity (Tripathy et al. 1996; Hampp et al. 1997; Volovik et al. 1999). Genomic and proteomic changes have been analyzed in spaceflight experiments. For example, in *Arabidopsis* callus 14d grown on board the Chinese spacecraft SZ-8, many key enzymes of the carbohydrate metabolic pathway, such as GDH1 and GDH2, were greatly upregulated, ensuring an effective cellular energy state during the adaption to microgravity (Zhang et al. 2015). The impact of microgravity on cellular trafficking and energy state might alter plant cell construction and metabolism. A thinner cell wall and decrease in cell wall constituents (polysaccharides) were observed under spaceflight conditions (Hoson et al. 2001; Soga et al. 2002; Nedukha 1997). Concerted changes in transcriptional and protein expression patterns and physiological traits have been noticed under long-term spaceflight stress. The expressions of cell wall-associated *CsExps* genes in cucumber seedlings peg were reduced 4-fold in microgravity compared with those in the ground control (Link et al. 2001). Lignin production is often reduced in spaceflight along with the activities of phenylalanine ammonia lyase and peroxidase, as particularly shown in pine seedlings (Cowles et al. 1984; Cowles et al. 1988). In *Brassica* stems, the concentration of 3-butenyl glucosinolate increased in orbit compared with ground controls.

Adaptation to Oxidative Stress

Removal of ROS species produced under spaceflight stress in plant cells is essential to minimizing oxidative damage to biological macromolecules. Reconstruction of antioxidant defense mechanisms to scavenge the ROS is key to plant adaptation to microgravity. Using transgenic *Arabidopsis* plants harboring the *alcohol dehydrogenase* promoter linked to a *β -glucuronidase* (*GUS*) reporter gene, Paul et al. (2001) showed that spaceflight affects stress-signal perception and transduction. The adaptive response was observed in altered expression of heat shock proteins (HSPs) in *Arabidopsis* cell cultures,HSP genes were induced exclusively in response to prolonged microgravity on board the ISS and under sustained clinorotation, but transient microgravity intervals in parabolic flight and various hypergravity conditions failed to induce them (Paul et al. 2005; Zupanska et al. 2013; Kozeko and Kordyum 2009).

Increases in Other Tropic Responses

Many plants can complete their life cycles in microgravity and spaceflight experiments (reviewed by Correll and Kiss 2008; Correll and Kiss 2011). According to early space experiments, gravity was not necessary to maintain normal growth and proper development. However, more recent studies have indicated that those results could be attributed to the flexible adaptation mechanisms of plants. Analyses of *Arabidopsis* plants carried by the space shuttle to the ISS demonstrated how gravity contributed to intrinsic growth patterns (Johnsson et al. 2009; Solheim et al. 2009). To compensate for the lack of gravitropism, plants under microgravity strengthened other tropic responses. For example, *Arabidopsis* hypocotyls display increased blue-light phototropism (Millar et al. 2010). Recently JAXA's HydroTropi experiments on the ISS showed that roots of cucumber seedlings became sensitive to moisture gradients, bending strongly toward moistened medium, and auxin-inducible genes such as *CsIAA1* and*CsPIN5* were expressed much more strongly in the responsive position of bending roots(Moriwaki et al. 2013).

Conclusions

Our knowledge of the short-term and long-term microgravity responses of plants in space has been greatly improved by recent advances in plant genomics and proteomics and in growth chambers allowing adequate environmental control for plant cultivation on space flights. Genome-wide DNA microarrays and global proteomic analyses have shown that, shortly after microgravity exposure, numerous genes are transiently up-regulated, many of which function in general stress responses, such as Ca^{2+}, lipid, and cytosolic pH signaling (Aubry-Hivet et al. 2014; Hausmann et al. 2014). However, after long-term acclimation, only a few genes remain at the elevated level and they are often functionally important for microgravity adaptation, probably via oxidative stress tolerance, metabolic changes, and tropic growth (Paul et al. 2005; Mazars et al. 2014; Zhang et al. 2015).

In the coming decade, plant research in space should consider the molecular regulatory network level, with emphasis on an advanced understanding of long-term microgravity effects and the molecular basis of plant adaptation to space. Plant growth facilities and sophisticated scientific equipment would be perfect for use in space. Genomic and proteomic studies will offer the promise of new data to better resolve the mechanism of plant responses and adaptation to short- and long-term microgravity. Long-term, uninterrupted, multigenerational, plant experiments will be possible on board the ISS and Chinese space station, which is expected to be fully operational by 2020.

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Conflict of interests The authors declare that they have no conflict of interest.

References

Andreeva, Z., Barton, D., Armour, W.J., Li, M.Y., Liao, L.F., McKellar, H.L., Marc, J.: Inhibition of phospholipase C disrupts cytoskeletal organization and gravitropic growth in Arabidopsis roots. Planta **232**, 1263–1279 (2010)

Aubry-Hivet, D., Nziengui, H., Rapp, K., Oliveira, O., Paponov, I.A., Li, Y., Hauslage, J., Vagt, N., Braun, M., Ditengou, F.A., Dovzhenko, A., Palme, K.: Analysis of gene expression during parabolic flights reveals distinct early gravity responses in Arabidopsis roots. Plant Biol. **16**, 129–141 (2014)

Babbick, M., Dijkstra, C., Larkin, O.J., Anthony, P., Davey, M.R., Power, J.B., Lowe, K.C., Cogoli-Greuter, M., Hampp, R.: Expression of transcription factors after short-term exposure ofArabidopsis thaliana cell culture to hypergravity and simulated microgravity (2-D/3-D clinorotation, magnetic levitation). Adv. Space Res. **39**, 1182–1189 (2007)

Barjaktarović, Ž., Schütz, W., Madlung, J., Fladerer, C., Nordheim, N., Hampp, R.: Changes in theeffective gravitational field strength affect the state of phosphorylation of stress related proteins in callus cultures of Arabidopsis thaliana. J. Exp. Bot. **60**, 779–789 (2009)

Barjaktarović, Ž., Nordheim, A., Lamkemeyer, T., Fladerer, C., Hampp, R., Madlung, J.: Time-course of changes in protein amounts of specific proteins upon exposure to hyper-g, 2-D clinorotation and random positioning of Arabidopsis cell cultures. J. Exp. Bot. **58**, 4357–4363 (2007)

Boonsirichai, K., Sedbrook, J.C., Chen, R., Gilroy, S., Masson, P.H.: ALTERED RESPONSE TO GRAVITY is a peripheral membrane protein that modulates gravity-induced cytoplasmic alkalinization and laterial auxin transport in plant statocytes. Plant Cell **15**, 2612–2625 (2006)

Boutté, Y., Grebe, M.: Cellular processes relying on sterol function in plants. Curr. Opin. Plant Biol. **12**, 705–13 (2009)

Briarty, L.G., Maher, E.P.: Reserve utilization in seeds of Arabidopsis thaliana germinating in microgravity. Int. J. Plant Sci. **165**, 545–551 (2004)

Briegleb, W.: Some qualitative and quantitative aspects of the fast-rotating clinostat as a research tool. ASGSB Bull. **5**, 23–30 (1992)

Claassen, D.E., Spooner, B.S.: Impact of altered gravity on aspects of cell biology. Int. Rev. Cytol. **156**, 301–373 (1994)

Chebli, Y., Geitmann, A.: Gravity research on plants: use of single-cell experiment models. Front Plant Sci. **2**, 56 (2011)

Correll, M.J., Kiss, J.Z.: Space-based research on plant tropisms. In: Gilroy, S., Masson, P.H. (eds.) Plant tropisms, pp. 161–182. Blackwell, Ames, USA (2008)

Correll, M.J., Pyle, T.P., Millar, K.D., Sun, Y., Yao, J., Edelmann, R.E., Kiss, J.Z.: Transcriptome analyses of Arabidopsis thaliana seedlings grown in space: implications for gravity-responsive genes. Planta **238**, 519–533 (2013)

Cowles, J.R., Lemay, R., Jahns, G.: Microgravity effects on plant growth and lignification. Astro. Phys. Lett. Commun. **27**, 223–228 (1988)

Cowles, J.R., Scheld, H.W., Lemay, R., Peterson, C.: Growth and lignification in seedlings exposed to eight days of microgravity. Ann. Bot. **54**, 33–48 (1984)

De Micco, V., Arena, C., Pignalosa, D., Durante, M.: Review. Effects of sparsely and densely ionizing radiation on plants. Rad. Env. Biophy. **50**, 1–19 (2011)

De Micco, V., De Pascale, S., Paradiso, R., Arone, G.: Microgravity effects on different stages of higher plant life cycle and completion of the seed-to -seed cycle. Plant Biol. **16**, 31–38 (2014)

Driss-Ecole, D.L.V., Carnero-Diaz, E., Perbal, G.: Gravisensitivity and automorphogenesis of lentil seedling roots grown on board the international space station. Physiol. Plant **134**, 191–201 (2008)

Fasano, J.M., Swanson, S.J., Blancaflor, E.B., Dowd, P.E., Kao, T.H., Gilroy, S.: Changes in root cap pH are required for the gravity response of the Arabidopsisroot. Plant Cell **13**, 907–921 (2001)

Fengler, S., Spirer, I., Neef, M., Ecke, M., Nieselt, K., Hampp, R.: A whole-genome microarray study of Arabidopsis thaliana semisolid callus cultures exposed to microgravity and nonmicrogravity related spaceflight conditions for 5 days on board of Shenzhou 8. BioMed Res. Int. Article ID 547495 (2015)

Geilfus, C.M., Mühling, K.H., Kaiser, H., Plieth, C.: Bacterially produced Pt-GFP as ratiometric dual-excitation sensor for in planta mapping of leaf apoplastic pH in intact Avena sativa and Vicia faba. Plant Methods **10**, 31 (2014)

Gillaspy, G.E.: The cellular language of myo-inositol signaling. New Phytol. **192**, 823–839 (2011)

Gjetting, K.S., Ytting, C.K., Schulz, A., Fuglsang, A.T.: Live imaging of intra- and extracellular pH in plants using pH usion, a novel genetically encoded biosensor. J. Exp. Bot. **63**, 3207–3218 (2012)

Hampp, R., Hoffmann, E., Schönherr, K., Johann, P., De Filippis, L.: Fusion and metabolism of plant cells as affected by microgravity. Planta **203**, S42–53 (1997)

Hausmann, N., Fengler, S., Hennig, A., Franz-Wachtel, M., Hampp, R., Neef, M.: Cytosolic calcium, hydrogen peroxide and related gene expression and protein modulation in Arabidopsis thaliana cell cultures respond immediately to altered gravitation: parabolic flight data. Plant Biol. **16**, 120–128 (2014)

Hejnowicz, Z., Sondag, C., Alt, W., Sievers, A.: Temporal course of graviperception in intermittently stimulated cress roots. Plant Cell Environ. **21**, 1293–300 (1998)

Herranz, R., Manzano, A.I., van Loon, J.J., Christianen, P.C., Medina, F.J.: Proteomic signature of Arabidopsis cell cultures exposed to magnetically induced hyper- and microgravity environments. Astrobiology **13**, 217–224 (2013)

Hoshino, T., Miyamoto, K., Ueda, J.: Automorphosis and auxin polar transport of etiolated pea seedlings under microgravity conditions. Biol. Sci. Space **18**, 94–95 (2004)

Hoson, T., Soga, K., Wakabayashi, K., Hashimoto, T., Karahara, I., Yano, S., Tanigaki, F., Shimazu, T., Kasahara, I., Masuda, D., Kamisaka, S.: Growth stimulation in inflorescences of an Arabidopsis tubulin mutant under microgravity conditions in space. Plant Bio. Supp. **1**, 91–96 (2014)

Hoson, T., Saiki, M., Kamisaka, S., yamashita, M.: Automorphogenesis and gravitropism of plant seedlings grown under microgravity conditions. Adv. Space Res. **27**, 933–940 (2001)

Hoson, T.K.S., Yamamoto, R., Yamashita, M., Masuda, Y.: Automorphosis of maize shoots under simulated microgravity on a three-dimensional clinostat. Physiol. Plant **93**, 346–351 (1995)

Im, Y.J., Phillippy, B.Q., Perera, I.Y.: InsP3 in plant cells. In: Munnik, T. (ed.) Lipid signaling in plants, pp. 145–160. Springer, Berlin, Germany (2010)

Inglis, P.W., Ciampi, A.Y., Salomão, A.N., Costa Tda, S., Azevedo, V.C.: Expression of stress-related genes in zebrawood (Astronium fraxinifolium, Anacardiaceae) seedlings following germination in microgravity. Gene. Mol. Biol. **37**, 81–92 (2014)

Johnsson, A., Solheim, B.G., Iversen, T.H.: Gravity amplifies and microgravity decreases circumnutations in Arabidopsis thaliana stems: results from a space experiment. New Phytol. **182**, 621–629 (2009)

Joo, J.H., Bae, Y.S., Lee, J.S.: Role of auxin-induced reactive oxygen species in root gravitropism. Plant Physiol. **126**, 1055–1060 (2001)

Kittang, A.I., van Loon, J.J., Vorst, O., Hall, R.D., Fossum, K., Iversen, T.H.: Ground based studies of gene expression in Arabidopsis exposed to gravity stresses. J Gravit. Physiol. **11**, 223–224 (2004)

Kliss, M., Macelroy, R., Borchers, B., Farrance, M., Nelson, T., Blackwell, C., Yendler, B., et al.: Controlled ecological life support systems (CELSS) flight experimentation. Adv. Space. Res. **14**, 61–69 (1994)

Kozeko, L., Kordyum, E.: Effect of hypergravtiy on the level of heat shock protein 70 and 90 in pea seedlings. Microgravitity Sci. Technol. **21**, 175–178 (2009)

Kriegs, B., Theisen, R., Schnabl, H.: Inositol 1,4,5-trisphosphate and Ran expression during simulated and real microgravity. Protoplasma **229**, 163–174 (2006)

Krinke, O., Novotná, Z., Valentová, O., Martinec, J.: Inositol trisphosphate receptor in higher plants: is it real. J. Exp. Bot. **58**, 361–376 (2007)

Leitz, G., Kang, B.H., Schoenwaelder, M.E., Staehelin, L.A.: Statolith sedimentation kinetics and force transduction to the cortical endoplasmic reticulum in gravity-sensing Arabidopsis columella cells. Plant Cell **21**, 843–60 (2009)

Link, B.M., Wagner, E.R., Cosgrove, D.J.: The effect of a microgravity (space) environment on the expression of expansins from the peg and root tissues of Cucumis sativus. Physiol. Plant **113**, 292–300 (2001)

Manzano, A.I., van Loon, J.J., Christanen, P.C., Gonzalez-Rubio, J.M., Medina, F.J., Herranz, R.: Gravitational and magnetic field variations synergize to cause subtle variations in the global transcriptional state of Arabidopsis in vitro callus cultures. BMC Genomics **13**, 105 (2012)

Martzivanou, M., Babbick, M., Cogoli-Greuter, M., Hampp, R.: Microgravity-related changes in gene expression after short-term exposure of Arabidopsi thaliana cell cultures. Protoplasma **229**, 155–162 (2006)

Matía, I., Van Loon, J.J.W.A., Carnero-Díaz, E., Marco, R., Medina, F.J.: Seed germination and seedling growth under simulated microgravity causes alterations in plant cell proliferation and ribosome biogenesis. Microgravity Sci. Technol. **21**, 169–174 (2009)

Mazars, C., Brière, C., Grat, S., Pichereaux, C., Rossignol, M., Pereda-Loth, V., Eche, B., Boucheron-Dubuisson, E., Disquet, I.L., Medina, F.J., Graziana, A., Carnero-Diaz, E.: Microgravity induces changes in microsome-associated proteins of Arabidopsis seedlings grown on board the international space station. Plos one **e91814**, 9 (2014)

Millar, K.D.L., Kumar, P., Correll, M.J., Mullen, J.L., Hangarter, R.P., Edelmann, R.E., Kiss, J.Z.: A novel phototropic response to red light is revealed in microgravity. New Phytol. **186**, 648–656 (2010)

Monshausen, G.B., Miller, N.D., Murphy, A.S., Gilroy, S.: Dynamics of auxin-dependent Ca2+ and pH signaling in root growth revealed by integrating high-resolution imaging with automated computer vision-based analysis. Plant J. **65**, 309–318 (2011)

Moriwaki, T., Miyazawa, Y., Kobayashi, A., Takahashi, H.: Molecular mechanisms of hydrotropism in seedling roots of Arabidopsis thaliana (Brassicaceae). Amer. J. Bot. **100**, 25–34 (2013)

Nasir, A., Strauch, S.M., Becker, I., Sperling, A., Schuster, M., Richter, P.R., WeiSSkopf, M., Ntefidou, M., Daiker, V., An, Y.A., Li X.Y., Liu Y.D., Lebert M.: The influence of microgravity on Euglena gracilis as studied on Shenzhou 8. Plant Biol. Suppl. **1**, 113–119 (2014)

Nedukha, E.: Effects of microgravity on the structure and function of plant cell walls. Int. Rev. Cytol. **170**, 39–77 (1997)

Paul, A.-L., Daugherty, C.J., Bihn, E.A., Chapman, D.K., Norwood, K.L.L., Ferl, R.J.: Transgene expression patterns indicate that spaceflight affects stress signal perception and transduction in Arabidopsis. Plant Physiol. **126**, 613–621 (2001)

Paul, A.-L., Popp, M.P., Gurley, W.B., Guy, C., Norwood, K.L., Ferl, R.J.: Arabidopsis gene expression patterns are altered during spaceflight. Adv. Space Res. **36**, 1175–1181 (2005)

Paul, A.-L., Wheeler, R.M., Levine, H.G., Jerl, R.J.: Fundamental plant biology enabled by the space shuttle. Amer. J. Bot. **100**, 226–234 (2013a)

Paul, A.-L., Zupanska, A.K., Schultz, E., Rerl, R.J.: Organ-specific remodeling of the Arabidopsis transcriptome in response to space flight. BMC Plant Biol. **13**, 112 (2013b)

Paul, A.L., Amalfitano, C.E., Ferl, R.J.: Plant growth strategies are remodeled by spaceflight. BMC Plant Biol. **12**, 232 (2012)

Paul, A.-L., Manak, M.S., Mayfield, J.D., Reyes, M.F., Gurley, W.B., Ferl, R.J.: Parabolic flight induces changes in gene expression patterns in Arabidopsis thaliana. Astrobiology **11**, 743–758 (2011)

Perbal, G., Driss-Ecole, D.: Mechanotransduction in gravisensing cells. Trends Plant Sci. **8**, 498–504 (2003)

Perbal, G., Jeune, B., Lefranc, A., Carnero-Diaz, E., Driss-Ecole, D.: The dose-response curve of the gravitropic reaction: a re-analysis. Physiol. Plant **114**, 336–42 (2002)

Perera, I.Y., Heilmann, I., Boss, W.F.: Transient and sustained increases in inositol 1,4,5-trisphosphate precede the differential growth response in gravistimulated maize pulvini. PNAS **96**, 5838–5843 (1999)

Perera, I.Y., Heilmann, I., Chang, S.C., Boss, W.F.: A role for inositol 1,4,5-trisphosphate in gravitropic signaling and the retention of cold-perceived gravistimulation of oat shoot pulvini. Plant Physiol. **125**, 1499–1507 (2001)

Plieth, C.: Calcium: just another regulator in the machinery of life. Ann. Bot. **96**, 1–8 (2005)

Poovaiah, B.W., Yang, T., van Loon, J.J.: Calcium/calmodulin-mediated gravitropic response in plants. J. Gravit. Physiol. **9**, 211–214 (2002)

Ruyters, G., Braun, M.: Plant biology in space: recent accomplishments and recommendations for future research. Plant Biol. Suppl. **1**, 4–11 (2014)

Salisbury, F.B., Bugbee, B.: Plant productivity in controlled environments. HortScience **23**, 293–299 (1988)

Salinas-Mondragon, R.E., Kajla, J.D., Perera, I.Y., Brown, C.S., Sederoff, H.W.: Role of inositol 1,4,5-triphosphate signalling in gravitropic and phototropic gene expression. Plant Cell Environ. **33**, 2041–2055 (2010)

Sato, E.M., Hijazi, H., Bennett, M.J., Vissenberg, K., Swarup, R.: New insights into root gravitropic signalling. J. Exp. Bot. **515**, 511–515 (2014)

Sato, F., Takeda, S., Matsushima, H., Yamada, Y.: Cell growth and organ differentiation in cultured tobacco cells under spaceflight condition. Biol. Sci. Space **13**, 18–24 (1999)

Smith, C.M., Desai, M., Land, E.S., Perera, I.Y.: A role for lipid-mediated signaling in plant gravitropism. Am. J. Bot. **100**, 153–160 (2013)

Soga, K., Wakabayashi, K., Kamisaka, S., hoson, T.: Stimulation of elongation growth and xyloglucan breakdown in Arabidopsis hypocotyls under microgravity conditions in space. Planta **215**, 1040–1046 (2002)

Soh, H., Auh, C., Soh, W.Y., Han, K., Kim, D., Lee, S., Rhee, Y.: Gene expression changes in Arabidopsis seedlings during short- to long-term exposure to 3-D clinorotation. Planta **234**, 255–270 (2011)

Solheim, B.G., Johnsson, A., Iversen, T.H.: Ultradian rhythms in Arabidopsis thaliana leaves in microgravity. New Phytol. **183**, 1043–1052 (2009)

Stanković, B., Volkmann, D., Sack, F.D.: Autotropism, automorphogenesis, and gravity. Physiol. Plant **102**, 328–335 (1998)

Stutte, G.W., Monje, O., Hatfield, R.D., Paul, A.-L., Ferl, R.J., Simone, C.G.: Microgravity effects on leaf morphology, cell structure, carbon metabolism and mRNA expression of dwarf wheat. Planta **224**, 1038–1049 (2006)

Takeda, S., Gapper, C., Kaya, H., Bell, E., Kuchitsu, K., Dolan, L.: Local positive feedback regulation determines cell shape in root hair cells. Science **319**, 1241–1244 (2008)

Tako, Y., Arai, R., Tsuga, S., Komatsubara, O., Masuda, T., Nozoe, S., Nitta, K.: CEEF, closed ecology experiment facilities. Gravit. Space Biol. **23**, 13–24 (2010)

Tan, C., Wang, H., Zhang, Y., Qi, B., Xu, G., Zheng, H.Q.: A proteomic approach to analyzing responses of Arabidopsis thaliana root cells to different gravitational conditions using an agravitropic mutant, pin2 and its wild type. Proteome Sci. **9**, 72 (2011)

Toker, A.: Phosphoinositides and signal transduction. Cell Mol. Life Sci. **59**, 761–779 (2002)

Toyota, M., Furuichi, T., Tatsumi, H., Sokabe, M.: Cytoplasmic calcium increases in response to changes in the gravity vector in hypocotyls and petioles of Arabidopsis seedlings. Plant Physiol. **146**, 505–14 (2008)

Tripathy, B.C., Brown, C.S., Levine, H.G., Krikorian, A.D.: Growth and photosynthetic responses of wheat plants grown in space. Plant Physiol. **110**, 801–806 (1996)

Vandenbrink, J.P., Kiss, J.Z., Herranz, R., Medina, F.J.: Light and gravity signals synergize in modulating plant development. Front. Plant Sci. **5**, 563 (2014)

Volovik, O.I., Kordyum, E.L., Guikema, J.A.: Some characteristics of photosynthetic apparatus under conditions of spaceflight. J. Gravit. Physiol. **6**, P127–128 (1999)

Wang, H., Zheng, H.Q., Sha, W., Zeng, R., Xia, Q.C.: A proteomic approach to analyzing responses of Arabidopsis thaliana callus cells to clinostat rotation. J. Exp. Bot. **57**, 827–835 (2006)

Wheeler, R.M.: Plants for human life support in space: from Myers to Mars. Gravit. Space Biol. **23**, 25–35 (2010)

Wolff, S.A., Coelho, L.H., Karoliussen, I., Jost, A.-I.K.: Effects of the extraterrestrial environment on plants: recommendations for future space experiments for the MELiSSA higher plant compartment. Life **4**, 189–204 (2014)

Wong, H.L., Pinontoan, R., Hayashi, K., Tabata, R., Yaeno, T., Hasegawa, K., Kojima, C., Yoshioka, H., Iba, K., Kawasaki, T., Shimamoto, K.: Regulation of rice NADPH oxidase by binding of rac GTPase to its N-terminal extension. Plant Cell **19**, 4022–4034 (2007)

Xu, D., Guo, S., Liu, M.: Identification of miRNAs involved in long-term simulated microgravity response in Solanum lycopersicum. Plant Physiol. Biochem. **66**, 10–19 (2013)

Zenko, C., Komatu, K., Yokoyama, R., Nishitani, K., Kamisaka, S.: Effect of hypergravity stimulus on XTH gene expression in Arabidopsis thaliana. Biol. Sci. Space **17**, 259–260 (2003)

Zhang, Y., Wang, L., Xie, J., Zheng, H.Q.: Differential protein expression profiling of Arabidopsis thaliana callus under microgravity on board the Chinese SZ-8 spacecraft. Planta **241**, 475–488 (2015)

Zheng, H.Q., Wang, H., Wei, N., Chen, A.D., Wang, L.F., Zheng, W.B., Zhang, T.: Live imaging technique for studies of growth and development of Chinese cabbage under microgravity in a recoverable satellite (SJ-8). Microgravity Sci. Tech. **20**, 137–143 (2008)

Zupanska, A.K., Denison, F.C., Ferl, R.J., Paul, A.-L.: Spaceflight engages heat shock protein and other molecular chaperone genes in tissue culture cells of Arabidopsis thaliana. Am. J. Bot. **100**, 235–248 (2013)