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Review

Recent transcriptomic studies to elucidate the plant adaptive response to spaceflight and to simulated space environments

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SUMMARY

Discovering the adaptation mechanisms of plants to the space environment is essential for supporting human space exploration. Transcriptomic analyses allow the identification of adaptation response pathways by detecting changes in gene expression at the global genome level caused by the main factors of the space environment, namely altered gravity and cosmic radiation. This article reviews transcriptomic studies carried out from plants grown in spaceflights and in different ground-based microgravity simulators. Despite differences in plant growth conditions, these studies have shown that cell wall remodeling, oxidative stress, defense response, and photosynthesis are common altered processes in plants grown under spaceflight conditions. European scientists have significantly contributed to the acquisition of this knowledge, e.g., by showing the role of red light in the adaptation response of plants (EMCS experiments) and the mechanisms of cellular response and adaptation mostly affecting cell cycle regulation, using cell cultures in microgravity simulators.

INTRODUCTION

Space exploration is one of the most fascinating enterprises for humans. It is becoming increasingly evident that plants are an essential companion of humans in space exploration as a privileged source of high quality nutrients and oxygen as well as waste recycling systems, that is, as a key component of bioregenerative life support systems (Fu et al., 2016). Thus, the search for the most efficient way of cultivating plants in space, including the knowledge of the mechanisms of adaptation of plants to the space environment and the careful selection of plant species, varieties and genetic lines, are current major challenges of plant space research (Medina et al., 2021). The most difficult parameter to overcome in the space environment is altered gravity level, because all other parameters such as oxygen and nutrient levels, humidity, temperature, or light conditions can be adjusted. Only cosmic radiation represents a challenge comparable to gravity because of the lack of 100% effective protective shields. On the other hand, plants, like every organism on Earth, have evolved under a constant gravity level and its modification or absence may be a difficult challenge to overcome for an organism.

There are different ways to study the effect of microgravity on an organism (Böhmer and Schleiff, 2019). Real microgravity research onboard the International Space Station (ISS) provides the most valuable information (Vandenbrink and Kiss, 2016), but it involves high cost and the access to it is very limited. Compared to a typical ground experiment, which usually involves practically unlimited sample size and the necessary number of performing personnel, spaceflight research imposes major limitations, including the size of and access to experiment equipment and the availability of trained staff and supplies needed to perform the scientific work. Furthermore, there are significant technical challenges that need to be overcome in the design and development of the experimental hardware necessary to operate in low gravity conditions. Retrieval, processing and storage of biological samples need to be optimized, in some cases by developing complex and costly procedures.

Despite all these constraints, six decades of research on the effects of long- and short-term missions to space have resulted in a consistent knowledge of many aspects of the impact of extraterrestrial factors, mainly exposure to microgravity, on biological systems. The story started with the sending to space of the first plant materials which were grown on unmanned vehicles. The Soviet Oasis 1 plant growth system,

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the first facility devoted to space plant research in history, was flown on the Cosmos 368 flight as early as 1970. Soon after, the facility was used by cosmonauts in the manned Salyut 1 mission in 1971 to grow different plant crop species. Since then, different plant cultivation experiments have been flown on crewed vehicles, including the Soviet/Russian Salyut and Mir, the American Shuttle, the Chinese Shenzou, and the ISS. Continuous subsystem improvements and increasing knowledge of plant responses to the spaceflight environment has led to the design of Veggie and the Advanced Plant Habitat, latest in the series of plant growth systems installed in the ISS (Ray et al., 2019; Zabel et al., 2016). All these steps were directed to achieve the main objective of spaceflight biology, which was to gain an understanding of adaptive processes triggered by terrestrial organisms when they are confronted with the novel space environment. The knowledge of the environmental limits of terrestrial biology in spaceflight is key to the design of successful space exploration strategies (Des Marais et al., 2008).

During spaceflight, exposure to a novel environment, previously unknown for terrestrial organisms, causes them to modify their growth and development patterns to a greater or lesser extent. These patterns are characterized by certain gene expression profiles capable of guiding growth and ensuring survival. During spaceflight, some sets of genes that were repressed in other environmental conditions are expressed and some other sets of genes are repressed in an attempt to cope with the new environment and adapt, or acclimate, to it (Paul et al., 2012). This response is not unique to plants, but it is enhanced in these organisms by the great reserve of plasticity exhibited by plant genomes, that enables them to respond to unforeseen environmental conditions, such as gravity alteration (Medina et al., 2021). In general, this plasticity is necessary because of 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 (Wang et al., 2011). For example, multiple cyclins and CDKs involved in cell proliferation control exhibit redundant mechanisms for producing the same effect in cell cycle checkpoints (Francis, 2011). In addition to gene redundancy, quicker mechanisms like transposable elements or epigenetic processes, such as DNA methylation operate under environmental stress (Lukens and Zhan, 2007).

This article revises the results of some relevant experiments performed in the ISS during the last decade and the knowledge acquired from them in terms of changes in gene expression at the global genome level (space omics). These changes are related to the acclimation or adaptation of plants to survive, grow and develop under the altered gravity conditions of extraterrestrial environments, either the microgravity characterizing spaceflight, or the partial gravity levels that can be found in the Moon and Mars surfaces. Together with gravity, another environmental stimulus is analyzed extensively, which is light. Both gravity and light are responsible for the two main tropisms that drive plant growth, namely gravitropism and phototropism. The regulatory effects of light and gravity on plant growth are closely related. An outstanding recent example of this relationship is the light regulation of the expression of *LAZY4*, a gene that plays an important role in the gravitropic response of both shoots and roots (Yang et al., 2020). In addition, upregulation of genes involved in a fundamental light-dependent function, such as photosynthesis, has been found as a response to microgravity condition in spaceflight, and, interestingly, this response occurred in etiolated plants, in the absence of any light signal (Kruse et al., 2020). The question on whether the deleterious effects of altered gravity could be compensated by light, or not, is critical for the success of plant culture in future human settlements on other planets, but attention to other suboptimal environmental parameters that may be unavoidable in spaceflight as cosmic radiation or reduced access to water or gas exchange may be also evaluated. In that regard, this review pays particular attention and its organized by the hardware used in each experiment, being crucial to understand the differential results observed in the "habitats" provided for each facility, in terms of suboptimal environmental conditions and particularly light.

The adaptive response to spaceflight environment of *Arabidopsis thaliana* seedlings under darkness condition (BRIC experiments)

A series of experiments called "Biological Research in Canisters" (BRIC) were performed in the NASA BRIC hardware, which consists of a simple compartment (canister) holding six 60-mm Petri dishes in small subcompartments called PDFUs (Petri Dish Fixation Unit cassettes) (Table S1). The PDFUs allow the sample fixation inside using an actuator gun. The BRIC hardware was flown as nonpowered payload so the seedlings

Table 1. Adaptive response to spaceflight environment of *Arabidopsis thaliana* seedlings under darkness

Conditions	Ecotype	Fixation	Tissue	Authors	GLDS
μg dark 12 days	Col-0	RNAlater	Seedlings	Paul et al. (2012)	GLDS-17
μg dark 12 days	Col-0	RNAlater	Seedlings	Kwon et al. (2015)	GLDS-44
μg dark 12 days	Ler-0	RNAlater	Shoot	Johnson et al. (2017)	GLDS-121
μg dark 8 days	Col-0, Ws-2, Ler-0 and Cvi-0	RNAlater	Seedlings	Choi et al. (2019)	GLDS-37
μg dark 3 days	Col-0	RNAlater	Seedlings	Kruse et al. (2020)	GLDS-38
μg dark 14 days	Col-0, *atire1*, *bzip60*, *bzip28*, *bzip28 bzip60*	RNAlater	Seedlings	Angelos et al. (2021)	GLDS-321

Experiments performed in BRIC hardware, indicating the main characteristics of each of them: experimental conditions, genotype, fixation, tissue used to carry out the transcriptomic study, authors and the GeneLab Data Set (GLDS). See also Table S1

were grown in the dark for the experiment duration, although a further development called BRIC-LED was implemented, providing lighting capabilities. In particular, BRIC-16 consisted of three individual experiments performed by three research groups as part of the STS-131 mission on the Space Shuttle Discovery in 2010. All BRIC-16 flight samples were grown in darkness for 309 h (12 days and 21h) and then fixed in RNAlater in-orbit. The Ground Control were BRICs prepared identical to spaceflight samples housed in Orbital Environmental Simulator (OES) at the Spaceflight Life Sciences Laboratory (SLSL-KSC) with a 24-h delay to enable duplication of the environmental parameters (temperature, humidity, and CO_2 concentration) collected by telemetry from the space shuttle (Johnson et al., 2017; Kwon et al., 2015; Paul et al., 2012) (Table 1).

The BRIC-16-DNA experiment (Impact of Spaceflight on *Arabidopsis*: Deep Sequencing and DNA Arrays as Collaborative Readouts of the Transcriptome of Arabidopsis Seedlings and Undifferentiated Cell in Space) disclosed that *A. thaliana* ecotype Columbia (Col-0) seedlings grown in space change the expression of 300 genes with respect to ground control samples. Between the differentially genes expressed, genes upregulated are associated with pathogen response, wounding, drought stress and cold stress (generalized stress-response) and genes downregulated are transcription factors involved in cell wall metabolism and cell elongation and genes encoding proteins that function in calcium-mediated signaling (gravitropism). The authors suggest that both the gravity signaling system and the stress signaling related to wounding and pathogens converge in their responses involving the actin-based cytoskeletal network attached to plasma membrane and the cell wall. It is possible that any of these signaling mechanisms are balanced by the force of gravity. Hence, the absence of gravity may confound the signaling system, inducing inappropriate stress responses as a consequence (Paul et al., 2012).

The transcriptional analysis of BRIC-16-Regulation experiment (Actin Regulation of *Arabidopsis* Root Growth and Orientation During Space Flight) showed that spaceflight conditions triggered the downregulation of genes involved in cell wall biogenesis and oxidative stress in Col-0 seedlings. Most of these genes are expressed in root hairs (class III peroxidase genes) independently of actin pathways because they were also downregulated in the other *Arabidopsis* line included in this experiment, the *act2-3*mutant. These gene expression changes led to shorter root hairs in space-grown seedlings. It is unclear whether this effect was due to microgravity *per se* or it was a consequence of other spaceflight environmental conditions producing stress, such as hypoxia, which produces the same phenotypic effect. Actually, the BRIC hardware system has several limitations: One is that the seedlings grow confined in small sealed containers for extended periods of time, and other is the absence of orbit 1*g* control which makes it difficult to distinguish true microgravity effects from other secondary spaceflight effects (Kwon et al., 2015).

BRIC-16-Cytoskeleton study (Investigations of the Plant Cytoskeleton in Microgravity with Gene Profiling and Cytochemistry) focused on gene expression changes in shoots of Ler-0 plants. The functional analysis showed that many upregulated transcripts in spaceflight conditions were implicated in metabolic process and transport. Most of these genes were localized in the extracellular matrix and involved in cell wall modification. On the other hand, and unlike the other two BRIC-16 experiments, the downregulated genes were often involved in stress response. This result was associated with the fact that the transcriptional analysis only included shoots. At the same time, an abundance of adventitious roots (immediately transport oxygen

from the substrate to shoots) was observed in spaceflight seedlings, suggesting that roots may undergo greater hypoxic stress than shoots during spaceflight. Therefore, pooling all plant organs together for a single analysis could provide an incomplete picture.

The BRIC-16 was a cluster of spaceflight experiments performed during the same space mission (STS-131) in the same hardware (Biological Research in Canister, BRIC) with the goal of studying the longer-term plant survival response in the spaceflight environment. Despite these common features, and even though transcripts within the same functional categories (stress response, defense, metabolic processes and cell wall modification) were found to be involved in the response in the different experiments, some differing results were obtained. These differences were associated to the specific experimental conditions of each one of the experiments: BRIC-16-DNA and BRIC-16-Regulation used the Col-0 ecotype as wild type whereas BRIC-16-Cytoskeleton used the Ler-0 ecotype; BRIC-16-Regulation and BRIC-16-Cytoskeleton growth media was half-strength MS salts and 1% (w/v) sucrose in agar, but BRIC-16-DNA used only half-strength MS salts in phytagel; BRIC-16-DNA and BRIC-16-Regulation used in microarray analysis RNA from entire seedlings whereas BRIC-16-Cytoskeleton used RNA from shoots only (Johnson et al., 2017).

The mission SpaceX CRS-4, launched on September 21, 2014, carried a new BRIC experiment with the aim of dissecting plant stress responses and revealing novel pathways for these responses triggered in space. RNA sequencing after growing for 8 days on board the ISS allowed the comparison of the transcriptomes of four *A. thaliana* ecotypes (Col-0, Ws-2, Ler-0 and Cvi-0) with ground-based control (a duplicated set of BRICs transferred to the ISS Environmental Simulator-ISSES with a 48-h delay to the flight experiment) (Table 1). Cvi-0 seedlings were those with the highest number of genes with deregulated expression, followed by Col-0 and WS-2 plants. In contrast, the Ler-0 plants barely showed changes in the expression of their genes. Common in all ecotypes, transcripts from heat-shock proteins (chaperones) were upregulated, whereas peroxidase transcripts were downregulated in spaceflight (Choi et al., 2019). According to the authors, this shared induction of molecular chaperones may help to protect cellular machinery from oxidative stress potentially triggered by hardware-dependent hypoxic response. In fact, alterations in growth and molecular responses in seedlings grown in BRIC have been described, raising the possibility that this hardware may impose intrinsic stresses on the plants and cause a response in some genes unrelated with the spaceflight environment (Basu et al., 2017; Johnson et al., 2017). The spaceflight ecotype-unique transcripts analysis showed that Cvi-0 was enriched in genes involved in light stress, kinase signaling and cell wall modification, Col-0 was enriched in transcripts related to cold and sugar response and, in Ws-2, enrichment in responses related to oxidative stress response was detected. Instead, Ler-0 also showed no significantly over-represented processes because of the small number of genes changing their expression. These results bring out significant differences between ecotype behavior, being Col-0 and Cvi-0 much more responsive to spaceflight conditions, whereas Ler-0 appeared to be relatively resistant to the same conditions, and therefore, highlighting the need to carefully consider the genetic background when comparing data sets and designing spaceflight experiments (Choi et al., 2019).

Another experiment that used the BRIC hardware to quantify protein and transcript differential expression of 3-day-old, etiolated Col-0 seedlings grown aboard the ISS, was launched on January 10, 2015 (Space-X CRS-5). The simultaneous proteomic and transcriptomic analyses provided a more complete picture of plant response to spaceflight (Kruse et al., 2020). In addition to the ground control (at ISSES with 48-h delay), in this experiment, two additional analyses were performed to eliminate significant changes in the transcriptome and proteome of *Arabidopsis* seedlings that could be attributed to the hardware (Basu et al., 2017) or to the RNAlater preservative (Kruse et al., 2017) (Table 1). These studies revealed marked impacts on post- transcriptional regulation, cell wall synthesis, redox/microtubule dynamics and plastid gene transcription in the plants exposed to microgravity. The effects on microtubule dynamics (alpha tubulins) were explained as a relevant physiological adaptation to the spaceflight environment, because alpha tubulin has the potential to enhance cytoskeletal organization and cell wall composition, thereby enhancing the structural integrity of cells in microgravity. A wide variety of plastid-localized and plastid-produced genes appeared differentially regulated in the flight samples, but they were not present in the proteomic data. This result was surprising because BRIC hardware prevents all light from reaching the plants, and it was interpreted as a wasteful effort to enhancing light signaling to compensate for the absence of gravity. In summary, the unified transcriptomic and proteomic analysis points out the regulation of specific elements of cell wall, microtubule synthesis and plastid transcripts production as three new targets to achieve optimal plant growth during future long-term spaceflight missions (Kruse et al., 2020).

During SpaceX CRS-12 mission (launched August 14, 2017) transcriptional responses of Col-0 seedlings and seedlings with a compromised unfolded protein response (UPR) to spaceflight were examined with the objective of studying how this response, which is a signaling cascade that responds to a number of unfavorable environmental and cellular stresses, could coordinate gene expression reprogramming in spaceflight stress conditions. For this, the BRIC-PDFU sterile plant culture hardware was used to grow for 14 days *Arabidopsis thaliana* wildtype and mutants defective in one or more components of the UPR, namely the TFs bZIP28 and bZIP60 (single and double mutants: *bzip28*, *bzip60* and *bzip28 bzip60*) and the ER-resident kinase/ribonuclease IRE1 (*atire1*). The transcriptomic results showed that growth in orbit substantially altered expression of thousands of genes associated with biological functions compared with ground control across all genotypes. Specifically, WT had the largest number of differentially expressed genes (1675 DEGs upregulated and 831 DEGs downregulated) and the bzip28 bzip60 mutant had the smallest number of DEGs (1293 upregulated and 562 downregulated). Among these DEGs, 34.8% (783/2249) of upregulated and 27.5% (335//1217) of downregulated overlapped across all genotypes whereas relatively smaller numbers of DEGs were found to be genotype-specific. Therefore, with the aim to gain more insights into the transcriptome changes caused by the absence of a correct UPR signaling under both ground and space flight conditions, the authors performed K-means clustering analysis on FPKM values for all DEGs. This analysis became clear that many of these spaceflight responsive genes were regulated uniquely in certain UPR mutants compared with WT in the ground control and that these variations in ground samples expression were largely muted by spaceflight. Therefore, a genotype-specific regulation in spaceflight condition was not observed. Overall, these results would suggest that the UPR does not have a broad involvement in the response to spaceflight but could play a partial role in regulating the transcriptional reprogramming in space compared with ground control. The reason could be that under space flight conditions many related stress-responsive genes were downregulated, being able to produce alternative signaling pathways, which repress the observed stress responses regulated by UPR (Angelos et al., 2021).

The adaptive response to spaceflight environment of *Arabidopsis thaliana* seedlings under light conditions

The TAGES (Transgenic Arabidopsis Gene Expression System) flight experiment, constituted by several independent runs launched to the ISS (Run 1: STS-129 November 16, 2009. Run 2: STS-130 February 8, 2010. Run 3: STS-131 April 5, 2010) was conducted within the ABRS (Advanced Biological Research System) hardware. This facility allowed to grow *Arabidopsis* seedlings on standard laboratory Petri plates and provided controlled lighting, temperature and air quality, and minimizing hardware-induced stress factors. In the three biological replicates, seedlings germinated, grew for 12 days and were harvested on orbit to RNAlater-filled KSC Fixation Tubes (KFTs). After returning to Earth, they were dissected into roots, leaves and hypocotyls for transcriptomic analysis (microarray). Ground control experiments (one per biological replicate) were conducted using identical ABRS hardware housed in OES chamber programmed to ISS environmental conditions (Paul et al., 2013) (Table 2). The microarray analysis showed that only 480 genes were differentially expressed in spaceflight samples in any organ, but most of these genes were not coordinately expressed among organs (only 26 genes were common in roots, hypocotyls and leaves). Although each organ displayed unique patterns of gene expression in response to spaceflight, the genes were involved in the same functional processes: the down-regulated genes were largely associated with factors regulating cell elongation and hormone signal transduction and the up-regulated genes were closely associated with pathogen responses, wounding and cell wall remodeling. Based on the results obtained, the authors pointed out that spaceflight environments had become extremely well controlled, there hardly being hardware-dependent factors, as indicated by the low number of genes with deregulated expression (48). Therefore, leaves, roots, and hypocotyls engage different genes in response to the spaceflight environment, but utilize similar adaptive strategies, consisting of initiating cellular remodeling and maximizing other environmental cues (for example, light) in a morphological response which is essential to produce a correct growth and development (Paul et al., 2013).

The CARA experiment (Characterizing *Arabidopsis* Root Attractions, launched on SpaceX CRS-3, April 18th 2014) consisted of growing three *Arabidopsis thaliana* genotypes (wild-types Wassilewskija (WS), Columbia-0 (Col-0) and phytochrome D mutant Col-0 PhyD (*phyD*)) on Phytagel plates for 11 days secured to a fabric support to the wall in the US lab of the ISS Destiny module. Some of them grew with the ambient light of ISS and some in the dark, wrapped in blackout cloth. After a growth period of 11 days, samples were harvested into KFTs containing RNAlater. A comparable set of plates, grown within the ISSES, monitoring temperature, CO_2, relative humidity and light levels, with a delay of 24 h after the initiation of the

Table 2. Adaptive response to spaceflight environment of *Arabidopsis thaliana* seedlings under light conditions

	Conditions	Ecotype	Fixation	Tissue	Authors	GLDS
ABRS	µg light 12 days	Adh:GFP, DR5r:GFP,35s:GFP	RNAlater	Root Leaf Hypocotyl	Paul et al. (2013)	GLDS-7
CARA	µg dark/ambient light 11 days	WS, Col-0, *phyD*	RNAlater	Root tips	Paul et al. (2017)	GLDS-120
SJ-10	1g 3 days 16h light µg 2,5 days 16h light	Col-0	RNAlater	Seedlings	Xu et al. (2018)	GLDS-220
	µg short-day long-day 48 days	Col-0, *pHSP:FT*, *pHSP:GF*	RNAlater	Rosette leaves	Xie et al. (2022)	NA
VPS	Mg constant light 4/8 days	WS, Col-0	RNAlater	Root	Beisel et al. (2019)	GLDS-218
	µg constant light 11 days	WS	RNAlater	Root Leaf	Zhou et al.(2019)	GLDS-217
	Mg constant light 4/8 days	Col-0, *spr1*, WS, *sku5*	RNAlater	Root	Califar et al. (2020)	GLDS-218
	Mg constant light 11 days	Col-0, *met1-7*, *elp2-5*	RNAlater	Root Leaf	Paul et al. (2021)	GLDS-416 GLDS-427
TG-2	µg short-day long-day 27 days	Col0, *pFT:GFP*, *ft-10*	RNAlater	Rosette leaves	Wang et al., 2022	NA

Experiments performed in different hardware that provided illumination, indicating the main characteristics of each of them: Experimental conditions, genotype, fixation, tissue used to carry out the transcriptomic study, authors and the GeneLab dataset (GLDS). See also Table S1

spaceflight experiment, was the ground control (Paul et al., 2017) (Table 2). The transcriptome responses in the root tips, which was the unique tissue used, showed that differentially expressed genes unique to each genotype were more abundant than those shared with another genotype. Under light exposure, Col-0 showed the greatest number of genes differentially expressed in spaceflight (297), *phyD* changed the expression of 130 genes and in WS only 71 genes were deregulated. Furthermore, each genotype used almost completely different sets of genes to physiologically adapt to the environment of the ISS. If this differential gene expression is considered as a measure of the physiological adaptive response cost, the WS ecotype would be better adapted to spaceflight than Col-0, and *phyD* mutation would be considered as a spaceflight-adaptive mutation in Col-0 background, suggesting that the plant genotype can be manipulated to reduce the metabolic cost of spaceflight adaptation. In the dark, WS and Col-0 seedlings showed roughly the same number of differentially expressed genes (218 and 224, respectively) and *phyD* exhibited the fewest genes with differential expression (131) indicating that the light environment affects physiological adaptation to spaceflight, which implies that the local habitat can be another factor that can be manipulated to reduce this cost. In this space experiment, all three genotypes were developmentally comparable to the ground control plants and they were equally productive in terms of root length so that only coordinately expressed genes shared amongst the genotypes (18 genes in light and 11 in dark) were considered fundamentally required for physiological adaptation of *Arabidopsis* to spaceflight. Most of them were upregulated genes associated with defense mechanisms or cell wall (Paul et al., 2017).

The RNA sequencing data collected from root tissue of Col-0 and WS seedlings grown in APEX03-2 (Advanced Plant Experiment 03–2) space experiment was used to investigate the role of alternative splicing during spaceflight adaptation in plants because it was described that terrestrial plant stressors arise unique alternative splicing patterns. In APEX03-2 experiment, launched on SpaceX mission CRS-5 on 10 January 2015, the seedlings grew on Phytagel Petri dishes kept in vertical racks perpendicularly oriented to the light source within the Vegetable Production System (VPS/VEGGIE) on board the ISS. The ground control was carried out inside the VPS ground unit within the ISSES chamber at KSC with a 48-h delay (Beisel et al., 2019; Ferl and Paul, 2016) (Table 2). Opposite to the pattern observed for differentially expressed genes, the analysis of the alternative splicing showed that fewer genes undergo spaceflight-induced significant differential alternative splicing in the Col-0 ecotype background (27) as compared to WS (48), with only

5 overlapping genes between them (Paul et al., 2017). Moreover, gene ontology analysis did not reveal any obvious conclusions respect to the function or localization of the genes with alternative splicing during spaceflight exposure. Although the detection of alternatively spliced transcript isoforms in spaceflight samples, regarding abundance, types, and functional implications, is still pending further investigation, this study presented strong evidence that alternative splicing plays a role in the process of molecular adaptation to the spaceflight environment in plants (Beisel et al., 2019).

The Advanced Plant Experiments 03–2 also studied the hypothesis that skewing pathways play a large role in spaceflight adaptation via cell wall remodeling. Differential gene expression profiles of two mutants, differing in their ecotype background, defective in two well-characterized genes which affect different skewing control pathways (*spr1* and *sku5*) and their wild type controls (Col-0 and WS) were examined after growing for 4 and 8 days on ISS. SPIRAL1 plays a role in directional cell expansion by regulating cortical microtubule dynamics and *spr1*mutation results in axial rotation of cell files throughout the plant, which manifests in roots as skewing to the left. SKU5 is a glycosylphosphatidylinositol-anchored cell wall and plasma membrane protein implicated in stress response signaling and *sku5*mutant exhibits rightward skewing (Table 2).Morphological data (angle between the root tip and the initial direction of root growth in the 4-day-old seedlings) showed that *spr1* seedlings exhibited a consistent leftward skewing phenotype on Earth that was substantially enhanced by spaceflight. Instead, *sku5*mutant showed a prevention of skewing in spaceflight compared to the ground control. The root length was not altered in either mutant line by spaceflight. The transcriptomic analysis revealed that in *spr1* seedlings the number of differential gene expression under spaceflight condition was less than in Col-0 seedlings. In addition, both lines differentially expressed fewer genes at the 8-day timepoint than at 4-day. In contrast, *sku5* seedlings the spaceflight differentially expressed genes were five-fold and two-fold more than WS at 4 days and 8 days, respectively. Also, both genotypes increase in their deregulated genes between 4 and 8 days.

Among genes that were upregulated in spaceflight for *sku5* many were LEA (Late Embryogenesis abundant) family genes, associated with seed dormancy, negative regulation of post-embryonic development and ABA signaling to enhance stress resistance (osmotic, cold, and drought) through membrane stabilization, ROS sequestration and protein aggregation prevention. Moreover, processes involved with salt, ROS stress, and light responses were terms enriched significantly at 4 days and at 8 days and terms associated with cell wall remodeling and biosynthetic processes were overrepresented.

The *sku5* and *spr1* different gene expression patterns, considering the number of differentially expressed genes and their fold-change levels as a measure of metabolic cost of adapting to environment, suggest that mutation of the SPR1 skewing pathway enhances spaceflight physiological adaptation and the SKU5 pathway plays a key role in adaptation to this environment. Furthermore, the fact that *spr1* and *sku5* root lengths are not altered by spaceflight, indicate that the effects of these mutations lie in processes directing morphology and structure and not in growth processes (Califar et al., 2020).

WS seedlings grown in the spaceflight experiment APEX03-2 for 11 days with constant light were used for the determination of differential methylation of leaves and roots (bisulfite DNA sequencing) and differential gene expression (RNA sequencing) in response to spaceflight (Table 2). The authors expected to elucidate the role played by the epigenome in the physiological adaptation to spaceflight. It is well documented that terrestrial biotic and abiotic stresses, such as pathogen attack, salt, and drought stress, are elicitors of differential DNA methylation (Zhou et al., 2019). Many genes of the networks involved in the response to these stresses are differentially expressed by plants in spaceflight. The results indicated that although large scale alteration of the average methylation level was not detected in CG, CHG and CHH contexts between flight and ground DNA, thousands of specifically differentially methylated cytosines were discovered with clear organ-specific patterns. In roots, hypomethylated CHH sites predominated whereas leaves showed a predominance of hypermethylated CHH sites. In leaves, about one-fifth of the genes differentially expressed in response to spaceflight (143 out of 743) were also differentially methylated (DmC-DEG), but in roots the fraction was about one-third (75 differentially expressed genes, 21 of which were also differentially methylated). Gene ontology analysis detected stress response to abiotic stimulus, temperature, radiation, and light stimulus categories in roots. In leaves, the detected categories included response to stressors of temperature, radiation and light, plus chemical stress, oxidative stress and a response to oxygen-containing compounds. In addition, in leaves, a 46% of DmC-DEG set were associated with reactive oxygen species (ROS). These results suggested that although DNA methylation may play a significant

role in a portion of the genes regulated by spaceflight, it is clearly not the only factor in this regulation. Furthermore, ROS genes may play a unique role in the adaptation of plants to the spaceflight (Zhou et al., 2019).

Furthermore, in "The Advanced Plant Experiment-04-Epigenetic Expression" experiment, launched on the SpaceX mission CRS-10 on 19 February 2017, two methylation mutant lines (*met1-7* and *elp2-5*) were grown on the ISS to assess their response to spaceflight compared to that of wild type (Col-0). The plants grew in the Veggie hardware (both on the ISS and in the ISEES chamber) with constant light conditions for 11 days, before being fixed in RNAlater into KFTs. Once on Earth, leaf and root tissues were dissected and, from each sample, whole-genome bisulfite sequencing (WGBS) and RNA sequencing (RNAseq) was performed (Table 2). The two mutant lines are deficient in separate and distinct functions that affect methylation. MET1 (methyl transferase) is directly involved in the maintenance (particularly at CG sites) of cytosine methylation in *Arabidopsis* and is an enzyme that modifies the epigenome, as part of stress responses. ELP2 (Elongator Protein) interacts with components of the siRNA machinery inducing cytosine methylation by recruitment of the DNA methyl transferase DRM2 and is associated with terrestrial pathogen responses, playing a role in root development (Paul et al., 2021). The results reinforced that, in overall average, genome methylation is not a hallmark of spaceflight adaptation in wild type plants because, for Col-0 plants, the degree of the genome-wide methylation was not statistically significant between spaceflight and ground control samples. Instead, both *elp2-5* and *mit1-7* plants were generally hypermethylated in spaceflight environment compared to the ground control. Each of the mutant lines demonstrated a different pattern of organ-specific expression and methylation. In *met1-7* plants, a large proportion of the differentially methylated and expressed genes in leaves was observed (72 out of 226), whereas in *elp2-5*, the large proportion of the differentially methylated and expressed genes (101 out of 120) was observed in roots, suggesting that MET1 plays a more important role in leaves and ELP2 in roots, in the process of physiological adaptation to spaceflight. The functional analysis showed that differential methylation took place in *elp2-5* line, together with the expression of gene classes that were associated with the spaceflight response in previous experiments, such as, metabolic processes and defense, hormone, and hypoxic responses. Besides, these same spaceflight-associated processes were also among the primary classes enriched in the spaceflight acclimation of Col-0. Conversely, the mutant *met1-7* line showed relatively few GO term enrichments, predominantly associated with signal transduction, as membrane transporters or transcriptional activators. These data suggest that *met1-7* plants were better adapted to spaceflight than *elp2-5*plants, and that the response to spaceflight environment acts more directly through mechanisms regulated by ELP2 than through those regulated by MET1 (Paul et al., 2021).

Epigenetic changes in Arabidopsis seedlings in response to spaceflight were studied by conducting a whole genome bisulfate sequencing to discover and qualitatively assess spaceflight-associated 5-methyl cytosine epigenetic modifications. In this experiment, 6- day-old Col-0 plants pre-cultured on ground were transferred to culture chambers aboard the Chinese recoverable scientific satellite SJ-10 (launched on April 6 and landed on April 18, 2016) and after grown up to 60 h (2.5 days) under microgravity conditions, seedlings were fixed with RNAlater using a canal system and pump support system included in the equipment.

The results demonstrated that lower methylation levels in CHG, CHH, and CpG context tended to occur under microgravity conditions. The molecular function analysis of differentially methylated regions (DMRs) showed that they correspond to genes involved especially in hormone signaling pathways including auxin, ABA, ethylene, and brassinosteroids, genes encoding transcription factors (TF) and genes associated with cell wall metabolism. In addition to methylation alterations at the gene level, transposon elements (TE) showed altered methylation levels during spaceflight. Overall, these results indicate that alterations in the methylation of auxin-related genes and cell wall may play important roles in plants adaptation to microgravity and DNA demethylation within TEs may cause transcriptional alterations in transposons during adaptation to spaceflight conditions (Xu et al., 2018).

Contrary to previous experiments that focus on studying the early adaptation response to the space environment, two Chinese missions whose results have been recently published, have focused on studying gene expression changes in the reproductive stage of *A. thaliana* plants. Because successful completion of the plant seed-to-seed cycle was considered essential to support human life in long-term manned missions, the main objective of these two experiments was to examine how two different photoperiod conditions could affect flowering time under microgravity. (Wang et al., 2022; Xie et al., 2022). One of the

experiments was carried out on board spacelab TG-2 (launched on September 15, 2016) in Plant Culture Box (PCB) hardware that controls the atmosphere, temperature, and hydrates the plants. Sterile seeds of WT (Col-0), *pFT:GFP* transgenic plants and *ft-10* were set on the panel root module containing commercially available vermiculite and stored dry until their hydration. The plants germinated and grew at 21°C under short-day (SD, 8h light/16h dark) or under long-day (LD, 16h light/8h dark) for 48 days, when one of LD PCBs with *Arabidopsis* plants was taken out by an astronaut and fixed with the RNAlater solution for transcriptomics analysis (Table 2). The initiation of bolting (start of peduncle growth) was delayed under the LD condition on board spaceflight (42 days after sown in spaceflight and 25 days after sown on ground control). According to this result, FT expression occurred later under this light condition because in the ground control the GFP fluorescence reached peak levels at 22 days after sowing and in microgravity the fluorescence peak was reached at 43 days after sowing. The microarray analysis performed using the leaves of WT plants grown under LD revealed that 1793 genes were upregulated whereas there were 1669 downregulated genes in the spaceflight samples with respect to their corresponding ground control. Among the DEGs, 53 flowering genes were identified and classified according to their known or predicted function in the flowering regulation pathway. The data showed that FT expression was upregulated along with a few genes involved in photoperiodism and circadian clock whereas the expression of SOC1 decreased accompanied by the downregulation of key genes involved in the response to the photoperiod, circadian clock, vernalization, ambient temperature, gibberellins and aging. These results indicate for the first time that FT and SOC1 could be key genes for *A. thaliana* plants to integrate spaceflight condition stress signaling into flowering regulation pathways (Xie et al., 2022).

The second experiment was carried out on the Chinese recoverable satellite SJ-10 (launched on April 6, 2016). The plant growth system used consisted of four growth compartments, illumination, photograph, air-flowing, heating, and humidity controlling system. The *A. thaliana* lines used were WT (Col-0) and transgenic plants (*pHSP:FT*, *pHSP:GFP* or FG) expressing FT together Green Fluorescent Protein (GFP) under control of a heat shock-inducible promoter (HSP17.4), by which it is possible to induce FT expression in-flight by heat shock treatment. The plants loaded in the growth chambers were germinated and grown in the root modules on ground under LD or SD conditions for 20 days, so they had about six to seven rosette leaves when the satellite was launched. After 12 days and 15 h in orbit, SJ-10 returned to Earth and 2 h after landing the plants were harvested and fixed with RNAlater solution (Table 2). As the images taken during the development of the experiment revealed, under the LD condition flowering occurred two days later in the space samples than in the ground controls for both the WT plants and the FG plants. In contrast, under the SD condition after heat shock treatment (37°C for 1h), in WT plants this delay was reduced to 1 day and in FG plants it disappeared, flowering both on ground and in space at 3 days. Microarray analysis was performed using the total RNA from leaves of WT and FG plants grown under LD and the SD in space and on ground, respectively. In the study of the categories of gene expression affected by spaceflight under different photoperiods, 4512 genes in WT and 4201 genes in FG response specifically to the LD or the SD (named "daylength-related-sp" or "dl-sp"). This higher number of "dl-sp" genes indicates that daylength is an important factor to regulate the response of plants to spaceflight. To further study response of plants to spaceflight after FT expression in the FG plants under the SD in comparison with WT plants, the authors compared the number of DEGs in response to spaceflight under the LD and the SD, respectively, among WT and FG samples. In these comparisons, they identified that 534 DEGS represented the group of genes in FG under the SD condition insensitive to spaceflight stress (unchanged in FG under the LD in space) and they were involved in cellular metabolic processes, as amino acid, amine, and oxoacid metabolic process.

Conversely, the most sensitive genes to spaceflight in FG plants under the SD condition (was significantly altered only in this condition) were 467 DEGs, many of them related with abiotic stimulus response and post-translational protein modification. Finally, 251 DEGs with function in chlorophyll biosynthetic pathway and response to stimulus non-changed their expression in WT under SD. These transcriptomic results together with bolting times indicated that heat shock-induced FT expression in FG plants could be used as a gen switch for flowering induction in space, which could change the sensitivity to the abiotic stimulus and/or cellular metabolic processes and therefore, alter plant response to spaceflight (Wang et al., 2022).

Gene expression changes in well-controlled environmental conditions including several light profiles and partial gravity levels (EMCS experiments)

The European Modular Cultivation System (EMCS) was another hardware facility aboard ISS which was used to grow plants on space. It provided directional light with white LEDs, controlled environment

Table 3. Adaptive response to spaceflight environment of *Arabidopsis thaliana* seedlings under light condition and partial gravity levels

Conditions	Ecotype	Fixation	Tissue	Authors	GLDS
1*g* white light 4 days µg, 0.07*g*, 0.13*g*, 0.21*g*, 0.39*g*, 0.53*g* and 1*g* red light 2 days	Ler-0	Freeze −80°C	Seedlings	Correll et al. (2013)	NA
1*g* white light 4 days µg, 0.09*g*, 0.18*g*, 0.36*g*, 0.57*g* and 1*g* blue light 2 days	Ler-0	Freeze −80°C	Seedlings	Vandenbrink et al. (2019); Herranz et al. (2019)	GLDS-251
µg and 0.36*g* white light 16h/dark 8h 4 days red light 2 days	Col-0	Freeze −80°C	Seedlings	Villacampa et al. (2021)	GLDS-314
1*g* (Ground Control) white light 16h/dark 8h 4 days red light 2 days	Col-0, *nuc1-2 nuc2-2*	Freeze −80°C	Seedlings	Manzano et al. (2020)	GLDS-313
µg, 0.53*g*, 0.65*g* and 0.88*g* constant light 5 days	Col-0	Freeze −80°C	Root	Sheppard et al. (2021)	GLDS-223

Experiments performed in EMCS hardware, indicating the main characteristics of each of them: experimental conditions, genotype, fixation, tissue used to carry out the transcriptomic study, authors and the GeneLab Data Set (GLDS). See also Table S1.

conditions (temperature, humidity, CO_2, O_2 and ethylene concentration) and it was equipped with two centrifuges that allowed to incubate the samples at different levels of gravity, up to 2*g*. This allowed them to perform an on-board 1*g* control, and also to incubate the samples at the so-called "partial gravity levels", lower than 1*g*, such as those characterizing the surfaces of the Moon and Mars. In the TROPI-2 experiment (launched on space shuttle mission STS-130 in February 2010) WT seedlings of *Arabidopsis thaliana Landsberg erecta* (Ler) ecotype were grown for 6 days within the EMCS, and they were photostimulated with blue or red light at seven gravitational acceleration levels (µg, 0.07*g*, 0.13*g*, 0.21*g*, 0.39*g*, 0.53*g* and 1*g*) for the last 48h of the growth period. For seedling growth, photostimulation and video recording, samples were contained in special cassettes equipped with a hydration mechanism remotely operated, blue, red and white LEDs, which were used instead of the EMCS lighting system, and an "anti-fog" device that prevented water condensation from the cassette window. After the photostimulation phase, a crew member transferred samples from the EMCS to the to the MELFI (Minus-Eighty Degree Laboratory Freezer for ISS) facility for preservation until download. The ground control was performed in a laboratory incubator following the exact timeline and light treatments as the spaceflight experiment (Kiss et al., 2012). TROPI-2 experiment was a duplication of the original TROPI experiment after the problems experienced with the samples because of the presence of toxic volatile substances in the hardware (Table 3).

The primary objectives of the TROPI-2 experiment were to study plant tropisms in spaceflight (interaction between gravitropic and phototropic signals) using images, but samples from this experiment were also used to perform transcriptome analyses with RNA was extracted from whole seedlings, with the purpose of maximizing the scientific returns of the spaceflight experiment. The comparison between all spaceflight samples, regardless of their gravity or light treatment, with the 1*g* ground samples, identified 280 genes that were differentially regulated. However, in the comparison of all *g*-treated spaceflight samples with microgravity samples, only 130 genes were identified as being differently regulated. From these genes, several of them were involved in cell polarity, cell defense or stress, and cell wall development. This result suggested that all of the spaceflight samples had a more similar environment because overall fewer number of genes were differentially expressed in the second comparison than in the first one, supporting the role that gravity plays in affecting these key developmental pathways, which were both represented in the two comparisons. In addition, as this hardware allowed performing a 1*g*-spaceflight control, these samples were compared with the 1*g* ground control. This resulted in a large number of genes differentially regulated (230), many of them implicated in cell-wall development, cell polarity and oxygen responses, among other categories. The fact that the 1*g* spaceflight *versus* 1*g* ground samples revealed many genes with deregulated expression could be because of spaceflight effects other than microgravity (vibration, radiation exposure, etc), emphasizing the need for tissue fixation on board the ISS and better environmental monitoring tools to improve ground experiments (Correll et al., 2013).

The Seedling Growth project (SG) was another plant space experiment performed in EMCS hardware, which consisted of three phases, each performed in different spaceflights: SG1 (uploaded via SpaceX CRS-2 in March 2013), SG2 (uploaded via SpaceX CRS-4 in September 2014) and SG3 (uploaded via SpaceX CRS-11 in June 2017). The goal of this project was to unraveling the link between the two main environmental cues driving plant development (light and gravity) at reduced gravity levels. Specifically, the project aimed at exploring if, and to what extent, specific light conditions could be applied to overcome, or at least to mitigate the deleterious effects caused by the lack of gravity on plant growth and development, thus facilitating the adaptation of plants to the space environment (Table 3). Ler seedlings grew under 6 nominal gravity levels (μ*g*, 0.09*g* or low gravity, 0.18*g* or Moon gravity, 0.36*g* or Mars gravity, 0.57*g* or reduced Earth gravity, and 1.0*g*) illuminated under white light for 96h (4 days) followed by 48h (2 days) of unidirectional photostimulation with blue light. The transcriptome analysis (RNAseq) of these samples revealed differential gene families affected and a clear difference in the intensity of the transcriptional response. At microgravity level photosynthesis, photosynthetic antenna proteins, porphyrin and chlorophyll metabolism, and protein processing in the endoplasmic reticulum were downregulated pathways. Nevertheless, only two pathways, ribosome synthesis and oxidative phosphorylation were found to be upregulated. These results suggested that a possible interplay between plant growth in microgravity and plastid function could exist (Vandenbrink et al., 2019). The analysis focused on partial gravity levels showed that as the gravity load increased the number of differentially expressed genes with respect to 1*g* control decreased, with a remarkable exception of the low gravity level (lower than 0.1*g*), which is the one that resulted in the highest number of deregulated genes. The DEG number was 296 in microgravity, 568 in low gravity, 123 in Moon gravity, 19 in Mars gravity and only 2 genes in reduced Earth gravity. In addition, the functional Gene Ontology (GO) analysis only identified enriched categories in μ*g*, low-*g* and Moon *g*-level, which were clearly different with the increasing partial-*g* level. In microgravity, light and photosynthesis GO terms were enriched, whereas response to stress, response to chemicals, and response to hormones were enriched in low gravity and the more representative enrichment in Moon gravity was related to cell wall and membrane structure. The general stress response detected in low gravity level was explained as the consequence of competition between phototropism (blue light) and the weak gravitropism signal (0.09*g*) to be the fundamental cue for driving seedling growth and plant development, resulting in a stress for the plant. Instead, the existence of the gravitropic response along with blue light illumination seemed to be enough to restore a nearly normal transcriptional state, particularly at Mars gravity level (Herranz et al., 2019).

The SG project also studied by transcriptomic changes the adaptive response of *A. thaliana* Col-0 seedlings grown at two nominal gravity levels (μ*g* and Mars gravity level, or 0.3*g*). These samples were illuminated for 4 days in a long-day photoperiod (16h white light and 8h dark) and in the last 2 days of growth period half of them were photostimulated with red light and the other half were kept in darkness (Table 3). The results indicated that only in microgravity condition, both in darkness and under red light, elevated plastid and mitochondrial genome expression was observed, being probably associated with a disturbed retrograde and anterograde communication between these organelles and the nucleus. Furthermore, in this condition, genes involved in hormonal pathways that promote proliferation, such as auxin and cytokinin signal transduction, were activated. On the other hand, at Mars *g*-level genes of hormone pathways related with stress response (abscisic acid, ethylene and salicylic acid) were activated. Furthermore, upregulation of environmental acclimation-related transcriptions factors (WRKY and NACs families) was also observed, especially in samples grown at Mars gravity level and photostimulated with red light, suggesting that seedlings grown in partial gravity level are able to acclimate by modulating genome expression in routes activated to cope with space environment-associated stress (Villacampa et al., 2021).

Under these same growing conditions, two knockout lines of nucleolin protein, *nuc1-2* and *nuc2-2*, affecting the two variants of this gene in *A. thaliana*, were grown in the SG series spaceflight experiments. The use of the nucleolin mutants can significantly contribute to the knowledge of the plant response to spaceflight environment, because NUC1 protein occupies a central position in ribosome biogenesis regulation, a complex stress-sensitive process which has been found to be seriously altered under conditions of spaceflight. In addition, *NUC1* gene expression is known to be upregulated by red light on Earth. In turn, NUC2 protein participates in the mechanisms of adaptive responses to different stresses. First, transcriptome analysis was performed only with ground control samples with the purpose of knowing the response of nucleolin mutants to different illumination conditions, namely red light and darkness (Table 3). Under red light photostimulation, ribosome biogenesis was the most significantly enriched category within the

upregulated genes *in nuc1-2 versus nuc2-2*. On seedlings kept in darkness during the last 2 days of cultivation, gene categories mainly involved in processes of cell division appeared promoted, and gene categories mainly related to developmental processes were downregulated in *nuc1-2* seedlings *versus* WT and *nuc2-2* seedlings. All these results indicate that on Earth the partial capability of the *NUC2* gene of replacing the functions of *NUC1* gene in darkness, when this gene is not present, is enhanced by red light. Furthermore, this photoactivation induces in the *nuc2-2*mutant an increased expression of genes belonging to functional groups associated to stress response, which means that NUC2 protein has a greater capacity of response to environmental signals, such as illumination with red light, and therefore, *nuc2-2* plants cannot develop a full response to this stimulus. The results of this work bring out, in space-omics spaceflight experiments, the need to pay special attention to the ground reference controls because identifying the genetic background in the this condition is essential before conclusions can be made on the spaceflight results (Manzano et al., 2020).

The "Plant Signaling" experiment, also carried out in the EMCS (launched in July 2011 aboard STS-135), studied the transcriptional profiling of root samples exposed for 5 days under continuous light at microgravity or partial *g* (0.53*g*, 0.65*g* and 0.88*g*) (Table 3). The analysis identified 101 genes whose expression levels were associated with gravity dose following three different patterns: increase or decrease expression in response to increasing *g* levels (dose-dependent linear response), peak or dip at an intermediate *g* level (peak up or peak down response) and induced or repressed expression levels to a threshold *g* level (threshold response). In addition, the identification of the biological functions or pathways of these 101 fractional-*g* responsive genes showed that they were genes involved in transcription regulation, chaperone function, cell wall and defense response. All of these categories had been previously described as being responsive to the plant response to the space environment (Sheppard et al., 2021).

Adaptive response to spaceflight environment of other plant species

The molecular response to the long-term spaceflight environment of Mizuna plants was measured using genome-wide mRNA expression analysis (mRNAseq). Seeds of *Brassica rapa var. nipposinica* (Mizuna) were germinated and grown in Lada growth chambers aboard to Zvezda module of ISS. These chambers include a root module, leaf chamber and light module. After 27 days cultivation, the plants were harvested and stored at −80°C in the MELFI freezer. Once back on Earth, total RNA from leaves samples was extracted.

The fresh weight and water content (spaceflight: 82.9g and 92.5%, ground control: 58.0g and 92.0%) showed that Mizuna plants grew as well or better under spaceflight environment than on the ground. The transcriptomic analysis revealed that in space-grown plants a total of 20 in 32 ROS oxidative markers were upregulated more than two-fold, including the major ROS-scavenging enzymes: thioredoxin and glutaredoxin. In addition, two ROS-producing genes (NADPH oxidase genes: RbohD and RbohF) were upregulated 3-fold and 5.1-fold, respectively. These findings suggest that the Mizuna plants can acclimate to long-term exposure in the spaceflight environment by reprogramming ROS gene network expression, producing ROS to maintain the activation of ROS signal network to control the defense system with ROS-scavenging system (Sugimoto et al., 2014).

Arabidopsis thaliana cell culture adaptive response to spaceflight environment (Table S2)

Arabidopsis thaliana wild type semisolid callus cultures were exposed to spaceflight conditions for 5 days within the Simbox hardware on board the Chinese spacecraft Shenzhou 8 (launched on October 31, 2011). The Simbox incubator enabled sample cultivation at controlled temperatures (22–24°C) and humidity (30–40%) and provided 1*g* in-flight control by means of an integrated centrifuge. In addition, this hardware included a pump system for metabolic quenching of the samples by RNAlater injection. The ground control experiment started with a one-day delay into the Simbox duplicate, according to the position in the flight incubator and kept at 23°C (Fengler et al., 2015) (Table 4). The microarray analysis detected 298 genes with differential expression between sample groups flight space (FS) and in-flight centrifugation (FC) but the differences in expression were not statistically significant. This result could mean that 5 days are enough for the culture adaptation to the new situation (spaceflight). Thus, in order to identify processes which are specifically influenced by microgravity and could provide important information about the physiological needs after a few days in space, overrepresented processes that were identical in the comparisons between FS samples and two 1*g* controls (in-flight centrifugation or FG and ground static or GS) were analyzed. Among the genes with upregulated expression, some of them constitute the ribosomal complex within plastids,

Table 4. *Arabidopsis thaliana* cell culture adaptive response to spaceflight environment

	Conditions	Organism	Fixation	Authors	GLDS
Simbox	µg dark 5 days	*Arabidpsis thaliana* (Col-0)	RNAlater (Simbox)	Fengler et al.(2015)	GLDS-213
	µg dark 324h	*Oryza sativa* (Rice)	RNAlater (Simbox)	Jin et al., 2018	GLDS-284
BRIC	µg dark 12 days parabolic flight simulated microgravity (2D clinorotation) simulated hypergravity (centrifuge)	*Arabidpsis thaliana*(Col-0)	RNAlater (PDFU)	Paul et al. (2012); Zupanska et al.(2013)	GLDS-17
	µg dark 10 days	*Arabidpsis thaliana* (Col-0, *arg1* and *hsfa2*)	RNAlater (PDFU)	Zupanska et al. (2017): Zupanska et al., 2019	GLDS-147 GLDS-205
	µg light (mid-deck) 1h, 8h and 20h	*Ceratopteris richardii* spores	Gaseous nitrogen freezer	Salmi and Roux (2008)	NA

Experiments performed in different hardware, indicating the main characteristics of each of them: experimental conditions, genotype, fixation, tissue used to carry out the transcriptomic study, authors and the GeneLab Data Set (GLDS). See also Table S2.

and some others were genes involved in electron transport chains located within mitochondria and representative for ATP biosynthesis. Among the downregulated genes the largest group was shown to code for HSPs. These results indicated that 5 days of exposure to microgravity did not cause detectable significant changes in gene expression, but caused an increased energy demand which should be taken into consideration when plants will be used as part of bioregenerative supports on long duration space missions. Finally, with the aim to separate responses to microgravity from those of non-microgravity-related spaceflight conditions, genes that were significantly altered within spaceflight samples (FS and FC) compared to the 1*g* ground control were studied. The majority of these genes were involved in the stress-induced antioxidant system, signaling chains, and defense-resistance response. The alteration of these processes was assumed as the effect of spaceflight-related environmental conditions, and especially, with the high radiation dose received by space samples (0.51 mSV/d), much more intense than terrestrial levels of radiation (1 to 2 mSV/a) (Fengler et al., 2015).

The aforementioned BRIC-16-DNA experiment also included the transcriptome analysis of *Arabidopsis thaliana* tissue culture cells (Paul et al., 2012)(Table 4). One of the most striking molecular differences observed was the significant increase in expression of most heat shock proteins (HSP) and heat shock factor (HSF) transcriptional activators genes in spaceflight non-differentiated tissue culture cells. Among these genes, the HsfA2 transcription factor which is involved in the late phases of heat stress response, and two of its targets: Hsp17.6A, that blocks protein aggregation, and Hsp101, that facilitates dissociation of already formed protein aggregates. In addition, the expression changes in these two HSP proteins, as markers of late stress response, were studied in parabolic flights (hypergravity and hypogravity alternation), in simulated microgravity (2D clinorotation) and in simulated hypergravity (centrifuge) by RT-qPCR. Among gravitational conditions examined, only 2D clinorotation induced the expression of both Hsp101 and HSP17.6A at the same levels as spaceflight. This fact suggests that hypergravity forces experienced at the space shuttle launch do not contribute to the elevated level of HSP transcripts in spaceflight samples. Therefore, a unique feature of microgravity engages HSPs as part of a later-phase adaptive response to spaceflight conditions, probably associated with the role played by them in cytoskeletal organization, preventing cytoskeletal scaffold collapse in a non-gravity environment (Zupanska et al., 2013).

The Cellular Expression Logic, or CEL BRIC-17 experiment, was a modification of the previous *Arabidopsis* cell cultures experiment in BRIC-16. In this experiment, launched on SpaceX CRS-2 to the ISS on 1 March 2013, besides the WT cell line, two mutant lines were used: ARG1 KO and HSFA2 KO (Zupanska et al., 2017, 2019). The knockout line deficient in the gene encoding ARG1 was selected for the role of this protein in gravisignaling, whereas the knockout line deficient in the gene encoding HSFA2 was selected because this protein is a key regulator of the defense response, via HSP chaperone transcriptional activation, to several types of environmental stresses (Table 4). The transcriptome analysis, after 10 days on orbit, showed

Table 5. *Arabidopsis thaliana* cell culture adaptive response to simulated space environments

	Conditions	Organism	Fixation	Authors	GLDS
Parabolic flight 2D Clinostat	0.16*g*, 0.38*g* and 0.5*g* dark 5min, 10min and 30min	*Arabidpsis thaliana* (Col-0)	RNAlater Freeze (liquid nitrogen)	Fengler et al. (2016)	NA
Levitation magnet Random Positioning Machine (RPM) Large Diameter Centrifuge (LDC)	µ*, 0,1*g**, 1*g**, 1.9*g** and 2*g** µ*g*2*g* dark 200min	*Arabidpsis thaliana*(MM2d)	Freeze (liquid nitrogen)	Manzano et al. (2012)	GLDS-8
Random Positioning Machine (RPM)	µ*g* dark synchronous subpopulations (enriched in G2/M and G1 phases) asynchronous population 14h	*Arabidpsis thaliana*(MM2d)	Freeze (liquid nitrogen)	Kamal et al. (2019)	GLDS-144

Experiments performed in different microgravity simulators or facilities, indicating the main characteristics of each of them: experimental conditions, genotype, and fixation, tissue used to carry out the transcriptomic study, authors and the GeneLab Data Set (GLDS). See also Table S3.

that both mutant cell lines used substantially more and different gene expression profiles to achieve a spaceflight-adapted state compared to WT cells. This observation led to conclude that a proficient spaceflight adaptation requires a functional HSF network and functional ARG1 protein. The functional gene categorization revealed that, although individual genes were different in each expression set, those genes were largely representative of the same biological processes, suggesting slightly different routes, which appeared to be utilized to converge on the same basic adaptive strategies. In addition, most of the genes altered in expression in spaceflight in WT cells were found to be *Arg1*- and *HsfA2*-dependent. Specifically, these genes were involved in cell wall remodeling, intracellular transport and starch biosynthesis, suggesting major and specific roles of these two proteins in the physiological spaceflight adaptation through these processes. Overall, this study showed up that the use of knockout lines greatly increases the list of genes that are necessary to adapt to and survive in the spaceflight environment. This amplifies the information that is necessary to learn on what biological processes are necessary and what genes are involved in these processes (Zupanska et al., 2017, 2019).

Arabidopsis thaliana cell culture adaptive response to simulated space environments (Table S3)

Cell cultures of *Arabidopsis thaliana* were exposed to partial-*g* forces (0.16*g*, 0.38*g* and 0.5*g*) for different times during parabolic flight (24 s, 33 s and 20 s) and clinostat experiments (5 min, 10 min and 30 min), in order to investigate gravity-dependent alterations in gene expression by whole-genome microarray analysis (Fengler et al., 2016) (Table 5). The results revealed that, in simulation by clinorotation, the least effects (the lowest number of differentially expressed genes at least 2-fold) were obtained after 30 min at 0.5*g*. Furthermore, the hierarchical clustering (Pearson's Correlation Coefficient) suggested that, in the clinorotation experiment, gene expression was more affected by the exposure time than by the change in gravity, because 0.16*g*, 0.38*g* and 0.5*g* generated no separate cluster for a given time of exposure and, interestingly, under 30 min of exposure, all gravity levels clustered with 1*g* controls. Although the clustering illustrated a low similarity between real (parabolic flight) and simulated partial-*g*, the screening at the single gene level identified many common differentially expressed genes, mainly at 0.38*g*. Among them, genes coding for proteins involved in cell wall modifications, peroxidases, auxin-related proteins, transmembrane proteins and components of intracellular signaling chains like different protein kinases, transcription factors and phytohormone-related proteins were identified. Taken together this study suggests that, after 30 min of clinorotation, specific responses are fading, demonstrating the insusceptibility of cell cultures over time and that clinorotation can induce changes in levels of transcripts similar to flight experiments (Fengler et al., 2016).

In another work, callus semi-solid cultures of *A. thaliana*, prepared from MM2d suspension cultures, were exposed for 200 min to two paradigms of simulated microgravity (a mechanical one, the Random Positioning Machine (RPM) and diamagnetic levitation) and hypergravity (the Large Diameter Centrifuge

(LDC)). Five environments with different levels of effective gravity (*g*) and magnetic field (B) strengths within a magnet (μ*g* *B = 10.1 T, 0,1*g** B = 14.7 T, 1*g** B = 16.5 T, 1.9*g** B = 14.7 T and 2*g** B = 10.1 T) and samples exposed to RPM simulated microgravity (μ*g*) and hypergravity (2*g*) in the Large Diameter Centrifuge (LDC) were compared (Manzano et al., 2012) (Table 5). The effects on the overall transcriptional state of callus exposed to these altered gravitational environments were studied. Microarray analysis indicated that barely a few genes were differentially expressed (not reaching statistical significance) when exposed to these unusual environments compared with internal 1*g* controls (particularly because of the large effect of high-energy magnetic field). However, using a detailed analysis based on clustering of similarly expressed genes (Gene Expression Dynamics Inspector-GEDI) it was found that gravitational and magnetic fields had a synergistic effect on the transcriptional profile because both the number of altered genes and the GO groups affected increased when a magnetic field gradient was applied, mainly affecting structural, abiotic stress and secondary metabolism genes. In fact, it is the first study in which a 0.1g partial gravity samples show larger effects that those observed in the equivalent simulated microgravity sample under the same environmental condition. The GEDI analysis also revealed that, there is a global signature, specifically including abiotic stress genes, that behave in an opposite manner under micro and hypergravity environments (Manzano et al., 2012).

Plant cell proliferation is a process known to be affected during spaceflight, but the molecular mechanisms involved in these changes remain unclear. To investigate transcriptomic changes in cell cycle regulation and progression by simulated microgravity, an *Arabidopsis* immobilized suspension culture was incubated in the RPM. Particularly, the analysis was performed with two synchronous subpopulations, enriched in G2/M and G1 phases, incubated for respectively 7 h and 14 h within the RPM, and an asynchronous reference cell culture incubated for 14h (Kamal et al., 2019) (Table 5). The results showed that the number of differentially expressed genes common in all populations by the simulated microgravity environment was low: 73 genes were upregulated, mostly being NADH/oxidative activity-related genes and mitochondria-related functions, and 83 genes were downregulated, most of them involved in ribosome activity and in photosynthesis-related functions. Conversely, simulated microgravity had differential effects on gene expression through the cell cycle progression. The expression of genes related to cell cycle regulation function, cell organization, ribosome biogenesis and developmental processes were downregulated in the G2/M population and upregulated in the G1 population, whereas abiotic stress response genes were upregulated in the G2/M population and downregulated significantly in the G1 population. Among the genes involved in abiotic stress response, genes that respond to UV-B, heat, osmotic shock and wounding are upregulated in synchronous G2/M population, but they were repressed in the synchronous G1 culture after 14 h of incubation in the RPM. These differential patterns of gene expression of stress pathways suggest differential sensitivity to microgravity of different phases of the cell cycle. It was proposed that a rapid initial response to a new environment within the first 7 h occurs mainly through the activation of expression of stress-related genes, whereas, at 14h of incubation, the gene expression patterns change as a result of a longer-term adaptive strategy that inhibits those pathways (Kamal et al., 2019).

Adaptive response to spaceflight environment of cell cultures from other species

Ceratopteris richardii spores were exposed to microgravity aboard NASA shuttle flight STS-93 to identify the early cellular and molecular events associated with gravity signal perception and response. These fern single-cells have been particularly valuable for investigating how cells sense and respond to gravity because this signal orients the polarity of their development shortly after light initiates their germination. The spores remained dormant in darkness inside BRIC canisters until the gravity force was less than 0.001*g*, and then were exposed to ambient light in the mid-deck to induce germination. After light exposure at three developmental time points (1 h, 8 h and 20 h) spores were frozen in a gaseous nitrogen freezer and were kept frozen until total RNA was isolated (Table 4). Microarray analysis showed that genes likely to encode peroxidases are downregulated in spaceflight development whereas protein phosphatase 2C and chaperone expression were upregulated in spores during the first 20 h of their germination in microgravity. Moreover, genes that may be involved in mobilization of reserve lipids were upregulated in spaceflight and genes whose products are likely involved in lipid hydrolysis/synthesis/transfer showed reduced mRNA levels during development in microgravity. These results, taken together, suggest that in microgravity lipids utilization and stress response changes occur in ferns and may be common features of spaceflight response in plant cells. Moreover, these findings provided new insights into early physiological responses to microgravity and are valuable data unraveling the basis genetic mechanisms that drive single-cell gravity responses (Salmi and Roux, 2008).

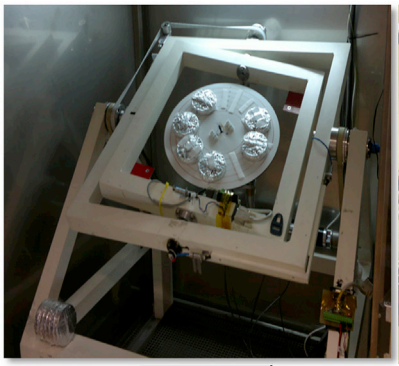
The spaceship Shenzhou mission also included one experiment to study gene expression in rice calli in response to microgravity. Rice calli (*Oryza sativa*) were loaded onto the stationary and rotating platforms of Simbox incubator and were fixed in RNAlater in space after 324h (13,5 days) spacecraft flight (Table 4). The functional analysis identified that the microgravity-response transcripts were associated with metabolism and signaling pathways of hormones, transcription factors, starch, glycolysis and the electron chain, protein degradation, transporters and cell wall metabolism. Among cell wall-related genes, genes related to cell wall formation/degradation were downregulated and transcripts corresponded to proteins involved in primary cell wall modification were upregulated. In addition, genes of the aquaporin family (water channels) were upregulated in spaceflight environment. The changed levels of these cell wall loosening and cell wall precursor synthesis-related genes together with aquaporin genes may establish of a new balance between cell wall rigidity and cell turgor pressure under microgravity conditions. Therefore, cell wall relaxation and extension may be regarded as microgravity plants adaptation (Jin et al., 2018).

DISCUSSION AND CONCLUSIONS

In the last decade, the landscape of plant biology research in spaceflight and simulated microgravity research has greatly advanced, mostly with the incorporation of space transcriptomics tools such as RNA-seq and microarrays. The aim of this review was therefore to revise the knowledge acquired through these techniques. The focus and organization of the work around the hardware used in the various experiments facilitated the comparison of the numerous results obtained and made it possible the identification of gene expression networks leading to a better knowledge of the plant adaptation mechanisms to spaceflight conditions. The report also highlighted the European contribution in the acquisition of this knowledge.

Even considering that novel experimental approaches take more time to be incorporated to space research than to general life science research on ground because of obvious and specific constraints of spaceflight experiments, these technologies have provided more than 25 datasets deposited in GeneLab database (Tables S1, S2 y S3). In that regard, Figure 1 illustrates the type of studies performed at the international level, arranged according to the materials and facilities used. The European contribution to the global plant biology experimentation is modest in the global numbers (8 of 30 studies) but particular contributions have been made in key topics. An example is the European co-leadership of the largest plant experiment carried out on board the ISS, using the most sophisticated hardware, that is the EMCS, including lighting at different wavelengths, partial-*g* and 1*g*, and environmental controls (Herranz et al., 2019; Vandenbrink et al., 2019; Villacampa et al., 2021). Also, there is a key contribution of European teams in simulated microgravity facilities and the use of cell cultures to uncover fundamental mechanisms discriminating the professional response to the lack of gravity from the purely cellular response and adaptive mechanisms. It is particularly relevant to realize that a small number of cell-based studies in simulators may have contributed to our understanding of these mechanisms more significantly than some spaceflight missions.

There is a clear dominance by number of studies in the use of BRIC hardware by NASA researchers (20% of the studies). This experimental hardware facilitates a large number of experiments to be quickly performed in orbit, but it shows serious limitations as to the environmental conditions that can be controlled and monitored. Indeed, the absence of a 1*g* control in space does not allow to discriminate between alterations caused by microgravity and other environmental factors such as hypoxia or a reduced confined space, which does not allow the cultivation of plants in good conditions over a period of time longer than a few days. For example, in the BRIC-16 experiments, the cytoskeleton was clearly shown to have a key role in the response of plants to spaceflight stresses, but it was not clear whether this response was a consequence of adaptation to the absence of gravity, or to another environmental factor independent of microgravity, such as hypoxia (Kwon et al., 2015). Other facilities with better control of the environmental conditions (ABRS and VPS/VEGGIE), particularly those including a 1*g* control on board (EMCS and Simbox harware), lead to more sophisticated results. In fact, the presence of a centrifuge on board not only makes it possible to obtain 1*g* control in space, and thus to discriminate the responses of plants because of the absence of gravity from other environmental factors, but it also makes it possible to obtain partial gravity levels such as that of the Moon (0.18*g*) and that of Mars (0.36*g*), which is essential for preparing future missions to the Moon and Mars. Improvements in cultivation constraints, optimization of controls and the systematic use of the model plant *A. thaliana* have allowed a great advance in the acquisition and comparison of results. It should be noted, however, that restoring terrestrial gravity or partial gravity such as that of Mars or the Moon on a centrifuge may induce experimental biases that have not yet been evaluated at the cellular scale. For example, the rotation may introduce vibrations to which the cells may respond independently of

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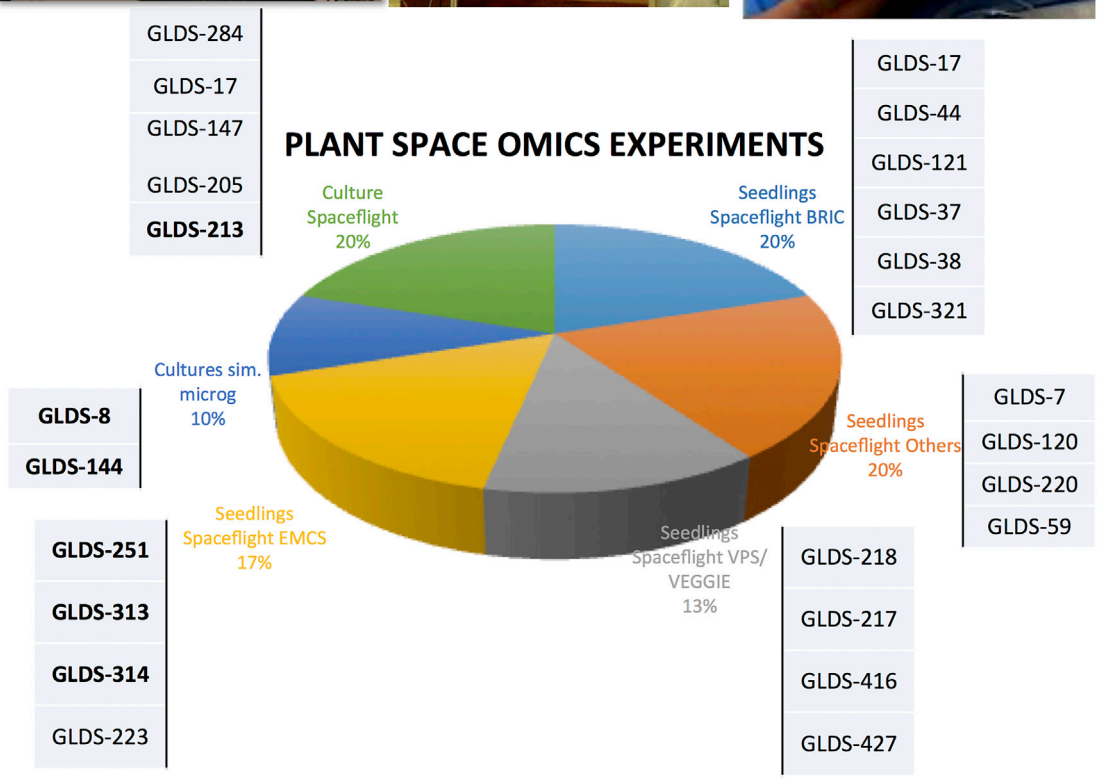
PLANT SPACE OMICS EXPERIMENTS
Culture Spaceflight 20%
Seedlings Spaceflight BRIC 20%
Cultures sim. microg 10%
Seedlings Spaceflight Others 20%
Seedlings Spaceflight EMCS 17%
Seedlings Spaceflight VPS/ VEGGIE 13%
GLDS-284
GLDS-17
GLDS-147
GLDS-205
GLDS-213
GLDS-17
GLDS-44
GLDS-121
GLDS-37
GLDS-38
GLDS-321
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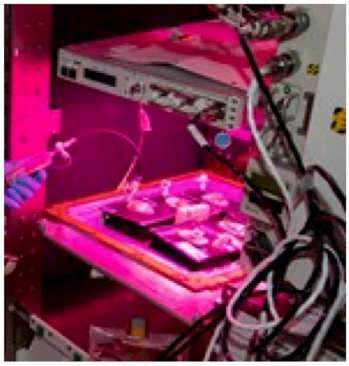

Figure 1. Distribution of Plant Space Omics contributions submitted to the GeneLab database

Most European contributions (in bold) have been done using cell culture experiments in simulated microgravity facilities (10% of the experiments) and the European Modular Cultivation System (EMCS) into the ISS (17% of the experiments). Other facilities frequently used in spaceflight experiments are the Biological Research in Canisters (BRIC) hardware and the VPS-VEGGIE cultivation chamber. At the top and the bottom part of the figure, images of some of the different facilities used are shown. From left to right and from top to bottom: Random Positioning Machine (credit ESA), Magnetic Levitation Instrument (credit Nottingham University) BRIC (credit NASA), Large Diameter Centrifuge (credit ESA), EMCS (credit ESA/NASA) and VEGGIE (credit NASA).

the level of gravity and the centrifugal forces are not applied homogeneously on a culture surface, but rather they are applied according to a gradient that is defined from the axis of rotation, which may be a source of some heterogeneity in the study population.

Despite the limitations in interpreting the results because of different experimental constraints, our knowledge of the adaptive response to spaceflight environment of *A. thaliana* seedlings has unequivocally advanced. Transcriptomic analysis techniques (RNAseq and microarray) as well as the consensual use of the model species *A. thaliana* allowed us to highlight common response pathways in the majority of the experiments carried out on board the ISS. Thus, the main processes affected identified in these studies were cell wall remodeling, oxidative stress, defense reaction, HSPs, and photosynthesis.

Consequently, we are in conditions of identifying some mechanisms of adaptation of plants to microgravity, based on similar gene expression profiles obtained in different experiments (Figure 2). The absence of gravity is perceived by the plant as a stress factor, which will trigger a whole series of adaptation reactions of the plant to this new environmental factor. Some of these reactions seem to be specifically targeted in the maintenance of cell integrity, such as cell wall remodeling, whereas others affect plant metabolism, such as photosynthesis, the electron transfer chain in mitochondria, and the communication between nuclei, plastids, and mitochondria. Finally, other reactions are systematically triggered by many biotic and abiotic stresses, such as oxidative stress, defense reactions and HSPs (chaperone proteins). Although the link between all responses has yet to be determined, it is clear that all of them play an essential role in the adaptation of plants to the space environment, becoming key targets for optimal growth plants to support future long-term spaceflight missions. (Figure 3).

Other molecular processes involved in the response to different environmental stress factors on Earth, such as DNA methylation and alternative splicing, seem to be also involved in this adaptation response, as the first transcriptomics studies of these two processes have revealed (Beisel et al., 2019; Paul et al., 2021; Xu et al., 2018; Zhou et al., 2019). However, different responses or even contradictory results depending on the environmental conditions have also been found between the different experiments (Johnson et al., 2017), strongly suggesting that the adaptive mechanisms, as a whole, should be more complex than these observed responses alone. In fact, the large number of different ecotypes and mutants used in the different experiments have shown that the response between the *Arabidopsis* ecotypes used is very different, some of them showing a greater capacity to adapt to the space environment or with less cost by changing the expression of a low number of genes, as it happens to Ler. (Choi et al., 2019; Paul et al., 2017). The mutant lines in different genes involved in various processes such as skewing, DNA methylation and UPR (Angelos et al., 2021; Califar et al., 2020; Paul et al., 2021) have provided more detailed information about the role that these processes play in the adaptation of plants to a space flight environment. In addition, differences between the genes whose expression is altered have been found between the different organs of the plant (Paul et al., 2013). Both findings indicate that the genetic background is an aspect to take into account to choice of the most suitable crop plant models and that knowing the integrated response of all parts of the plant will be crucial for the next phase of spaceflight experimentation.

Light, as the results of the different experiments demonstrate, is an essential factor in all plant development stages under spaceflight environment. The light quality, and specifically red light in the early stages of development (seedlings), modulates the expression of genes involved in related-stress pathways to cope with environmental spaceflight conditions (Villacampa et al., 2021). In addition, the day length is a factor that can regulate the plants flowering time, and it is even possible to use a genetic switch to induce flowering by adding a treatment, such as a heat shock (Wang et al., 2022; Xie et al., 2022). Furthermore, it has been shown that the adaptive response of plants depends on both the level of gravity (Herranz et al., 2019; Sheppard et al., 2021)) and the time of exposure to the spatial environment (Fengler et al., 2015; Kamal et al., 2019)). In fact, an early and a late response can be identified in the adaptive response of plant cell

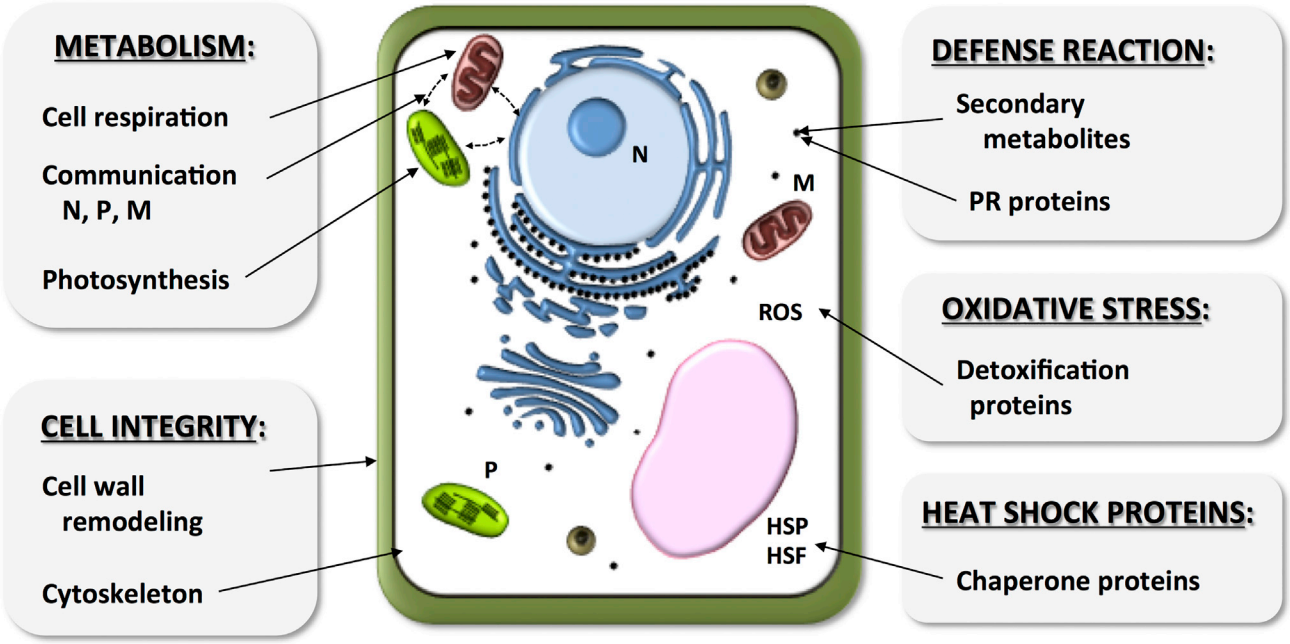

Figure 2. The main spaceflight-related genes

Transcriptomic analyses of the numerous space experiments have so far identified a set of genes whose expression is altered by the spaceflight environment in a similar way among the different experiments. The genes were grouped according to the function of their proteins. (N) nucleus, (P) plastid, (M) mitochondria, (PR protein) pathogenesis related protein, (ROS) reactive oxygen species, (HSF) heat shock factor, (HSP) heat shock protein.

cultures grown in simulated microgravity (Kamal et al., 2019). ROS production was also demonstrated in other members of the *Brassicaceae* family, such as Mizuma (*Brassica rapa*) and parietal changes were observed in rice (*Oryza sativa*), confirming the key role of these two cellular processes in the adaptation processes of plants to microgravity.

Finally, we consider it necessary to recapitulate why this research is actually important and worth to be performed. As previously indicated, studying the adaptation of plants to the space environment is an essential step towards successfully integrating plants into life support systems. During spaceflight, plants will have to adapt to a new environment where factors such as micro- and mini-gravity (gravities of spaceflight, the Moon and Mars) and chronic low-level cosmic radiation are completely new to them. In that regard, experiments introducing partial gravity levels, and particularly the levels around the Moon gravity, are becoming mandatory. A potential risk in using low gravity levels (*circa* 0.1*g*) with more deleterious effects than microgravity itself have been revealed (Herranz et al., 2019; Manzano et al., 2018), and the decommissioning of EMCS in 2018 from the ISS made us less able to provide new experiments at those conditions.

In this field, transcriptomic studies are a privileged strategy because they allow an analysis of gene expression in its entirety, leading to the identification of response pathways that would not be detected by more targeted approaches (Lowe et al., 2017). They also have the advantage of requiring only a relatively small quantity of transcripts (1 ng of total RNA for RNAseq and 1 μg of mRNA for microarray), which is a major advantage in the context of space experiments where the quantity of biological material is always very limited. In contrast, there are two potential caveats to the advance of the research field. The first is the uneven distribution of spaceflight opportunities in space agencies, particularly the unavailability of ESA to provide a replacement with similar capabilities to EMCS. The second is the analysis and integration of results from so different experiment platforms, materials and designs. New international consortia have been created to cope with better tools for transcriptomic analyses, improvement to the access of datasets and metadata, contributing decisively to the optimization of the new experiments to be conducted in the near future. GeneLab (Overbey et al., 2021), TOAST (Barker et al., 2020), ESA Space Omics TT (Madrigal et al., 2020) and ISSOP (Rutter et al., 2020) are highly relevant initiatives in this direction.

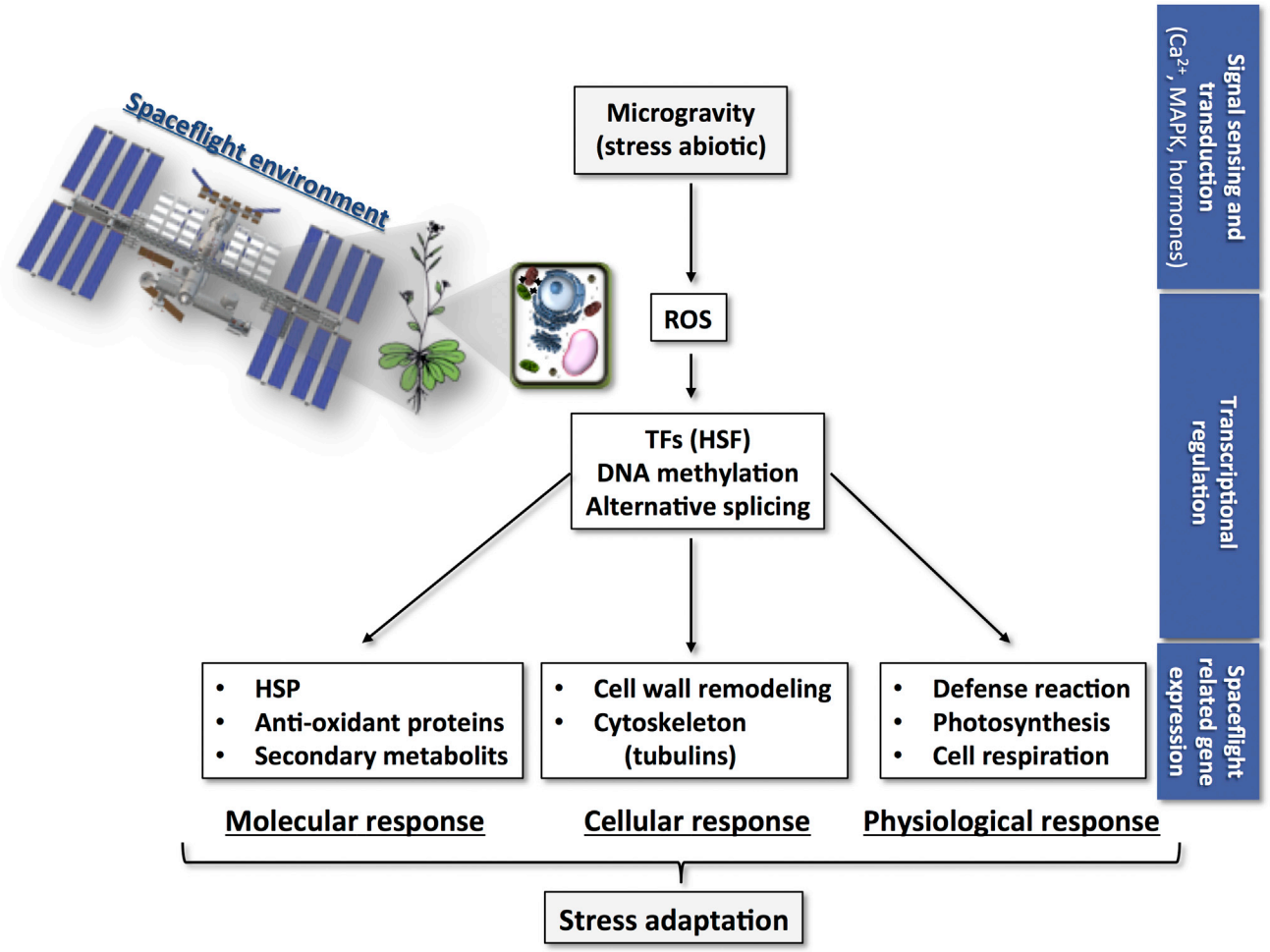

Figure 3. Proposed model of the microgravity adaptation mechanism in *Arabidopsis thaliana*

Microgravity is perceived by the plant as an abiotic stress factor which activates the production of ROS. The signal, which is perceived at the plasma membrane, is then transmitted via Ca2+, MAPKs and phytohormones (auxin) to the nucleus where it triggers TFs like HSF. The TFs then regulate the expression of a set of genes that trigger molecular, cellular and physiological responses, leading to the establishment of the plant adaptation to the spaceflight environment. (ROS) reactive oxygen species, (TFs) transcription factors, (HSF) heat shock factor, (HSP) heat shock protein.

Limitations of the study

The transcriptomic studies included in this review focus on the search carried out mainly in GeneLab Data Repository and despite the fact that the GLDS data set includes an interface to search datasets on key repositories (NIH GEO, EBI PRIDE, and ANLMG-RAST), some work focused on transcriptomic changes produced by a space environment might not have been included.

Methods details

To locate the works that study the plants adaptive response to a real or simulated space environment through transcriptomic analysis, NASA GeneLab data repository has been used as a reference (https://genelab.nasa.gov/). To perform an exhaustive search, the word 'plants' was used as a filter in the search data box and the total results were checked one by one to confirm that the publication includes and meets the criteria. In addition, to search for works not included in this database a manual search was carried out, in the Web of Science, Google Scholar and PMC-NIH using as key search terms 'plants spaceflight'/ 'plants simulated microgravity' together with 'RNA sequencing ', 'microarray', 'transcriptomic', 'epigenomics', and 'alternative splicing'.

SUPPLEMENTAL INFORMATION

Supplemental information can be found online at https://doi.org/10.1016/j.isci.2022.104687.

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AUTHOR CONTRIBUTIONS

Conceptualization and Investigation, AM; Writing-Original draft, AM and FJM; Writing-Review and Editing, AM, ECD, RH and FJM; Visualization, AM, ECD and RH; Funding Acquisition, ECD, RH and FJM.

DECLARATION OF INTEREST

The authors declare no competing interests.

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