DNA microarray analysis of gene expression of etiolated maize seedlings grown under microgravity conditions in space: Relevance to the International Space Station Experiment “Auxin Transport”

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Abstract
This paper introduces the use of microarray data technology with the Agilent Maize Oligo Microarray (Design ID 016047) to characterize global changes in the transcript abundance of etiolated Zea mays (cv. Golden Cross Bantam) seedlings grown under microgravity (μg) conditions on the International Space Station (ISS) compared with those grown under 1 g conditions on Earth. Gene array data were analyzed according to stringent criteria that restricted the scored genes for specific hybridization values at least two fold. Of the 32152 - 32616 transcripts detected, 1030 and 590 transcripts were significantly different in the coleoptiles and in the mesocotyls. Of the transcripts detected, 877 and 428 transcripts were found to increase under μg conditions in the coleoptiles and the mesocotyls, respectively. Venn diagram analysis showed that 154 transcripts commonly increased and 10 decreased under μg conditions irrespective of the organ difference. Of these, phytohormone-related genes were focused, indicating that some of them were responsive to gravity. These results support the commonly accepted idea that phytohormone-related genes play a significant role in regulating plant growth and development under different gravity conditions.

Keywords: Auxin-related gene, International Space Station experiment; Microarray; Zea mays

Introduction
Plant growth and development are substantially affected by various environmental factors. Of these, gravity is one of the most important environmental factors in regulating the physiological processes in the life cycle of a sessile plant on Earth. In order to clarify how gravitational stimuli affect the growth and development of plants, microgravity (μg) and hypergravity conditions have become novel and powerful tools. In particular, studies of growth response, physiological responses, and changes in phytohormone status in plants grown under μg conditions in space have been done (Halstead and Dutcher, 1987; Kamada et al., 2000; Correll and Kiss, 2008; Hoson et al., 2009, 2014; Paul et al., 2013; Kiss, 2014; Wakabayashi et al., 2015, 2017; Ferl et al., 2016; Yamazaki et al., 2016; Johnson et al., 2017; Soga et al., 2018; Ueda, 2020). Under μg conditions in space, plants have exhibited endogenously directed spontaneous growth called automorphogenesis (see review of Stanković et al., 1998).

The “Auxin Transport” (NASA’s nomenclature) space experiment was conducted in 2016 and 2017 in the Japanese Experiment Module (JEM) on the ISS, with the objective of obtaining integrated analyses of the growth and development of etiolated pea (cv. Alaska) and maize (cv. Golden Cross Bantam) seedlings in space relative to
polar auxin transport (PAT) (Ueda, 2020). Some of the relevant molecular mechanisms showing an important role that PIN proteins play as efflux carriers of auxins in gravity-controlled PAT have been reported (Miyamoto et al., 2019; Kamada et al., 2019, 2020; Oka et al., 2020). The PAT of etiolated pea and maize shoots grown under μg conditions in space was substantially decreased and enhanced, respectively, compared with those grown on artificial 1 g conditions on the ISS or 1 g conditions on Earth as observed in the BRIC-AUX experiment on the STS-95 mission using the shuttle Discovery (Ueda et al., 1999, 2000). These results clearly show that gravistimulation can positively and negatively regulate PAT in etiolated pea and maize seedlings, respectively. We have already reported that the mechanism of μg-enhanced PAT in maize shoots is most likely due to the enhanced ZmPIN1a accumulation and altered ZmPIN1a localization in parenchymatous cells of the coleoptiles (Oka et al., 2020); however, the mechanisms that enable the cells to acclimate to the changing gravistimulation are still poorly understood.

To clarify the global changes in transcript abundance elicited by various perturbations of μg conditions in space, we introduced microarray data technology with microarrays of Medicago (Medicago truncatula) to etiolated Alaska pea seedlings grown under μg and artificial 1 g conditions on the ISS; we found six auxin-related genes that are regulated by gravity. Furthermore, expression of several water channel genes was found to be gravity-regulated (Kamada et al., 2020). Yet, less information is available as to which genes are involved in growth and development of etiolated maize seedlings grown under μg conditions in space.

Based on this information, we introduced microarray techniques (the Agilent Maize Oligonucleotide Array, design ID 016047 from Agilent Technologies in the USA) to clarify gene expression profiling in the coleoptiles and the mesocotyls of etiolated maize seedlings grown under μg conditions on the ISS, comparing them with profiling of those grown under 1 g conditions on Earth. Novel findings on genes related to phytohormones, water channels, and others could significantly improve our understanding of how growth and development of the etiolated maize seedlings are regulated in space. Possible modes of action of these genes on the growth and development of etiolated maize seedlings grown under μg conditions in space will be briefly discussed.

Materials and Methods

Outline of the space experiment “Auxin Transport”

The “Auxin Transport” (NASA’s nomenclature) space experiment consisted of four runs with pea seedlings (Runs 1, 2, and 3) and maize (Run 4), and was conducted in 2016 and 2017 in the Japanese Experiment Module (JEM) on the ISS. The second experiment, Run 4 with maize and Run 1 with peas, launched on the Space X-10 mission in February 2017. It was conducted in the ISS Increment 50 in March 2017. The samples were returned to Earth in March 2017 by the Cargo Dragon capsule carried aboard the Space X-10. Details of the “Auxin Transport” experiment have already described in our previous manuscripts (Miyamoto et al., 2019; Ueda, 2020).

Plant materials

Maize (Zea mays L. cv. Golden Cross Bantam) was used in the ISS space experiment. As the seed bed, dry rockwool block (the thickness: 32 mm; Culture Mat; Nippon Rockwool, Tokyo, Japan) was fitted in an acrylic resin maize box (W 62 mm × D 100 mm × H 150 mm), which had six holes (1 cm in diameter) and covered with the hydrophobic fluoropore membrane, MilliSeal (Merck Millipore, Tokyo, Japan) for ventilation was used. Twenty dry maize seeds were inserted beneath the block surface with the seed axis longitudinal to the maize box. After an astronaut supplied 120 mL of water (Milli-Q water, autoclaved) to each maize box, the boxes were kept in the Measurement Experiment Unit, and then placed in the μg compartment in an incubator, the Cell Biology Experiment Facility (CBEF). The maize seeds were allowed to germinate and grow at 25°C in the dark for four days (96 h 27 m) under μg conditions in space. A part of the maize seedlings was used for determining the PAT, for the immunohistochemical analysis of ZmPIN1a proteins, and for the western blotting analysis of ZmPIN1a proteins.

The remaining maize seedlings were frozen at −95°C in the Minus Eighty-Degree Celsius Laboratory Freezer for ISS (MELFI) for later analysis of gene expression. These samples were returned to Earth by the Cargo Dragon capsule and sent to our laboratory; their storage temperature maintained with dry ice. The frozen seedlings were then stored at −80°C until RNA extraction.

It is impossible to use the full capacity of the CBEF or a 1 g centrifuge for this maize experiment, because the maize box is too large to fit into the CBEF. The 1 g control experiments were done at JAXA’s Tsukuba Space Center in Japan according to the procedures and the ISS experiment schedule (Miyamoto et al., 2019). Using a plant growth chamber (LPF-241SPC, Nippon Medical & Chemical Instruments, Co., Ltd., Osaka, Japan), the temperature, humidity, and CO2 concentration were adjusted to the environment of the ISS experiment. The CO2 concentration was about 3,500 ppm during experiment. Other experimental conditions were same as those used in the space experiment.

Extraction of total RNA

Total RNA was extracted using the methods reported by Kamada et al. (2019) with some modifications. The coleoptile region (from 3 to 8 mm below the tip) and the mesocotyl region (from 1 to 6 mm below the node) were carefully excised on the dry ice from the frozen etiolated maize seedlings of the ISS experiment and in the control experiment on Earth. A set of three coleoptile or mesocotyl segments was homogenized for 20 seconds with beating at 5,500 rpm four times in a Micro Smash MS-100 bead beater (Tomy Seiko, Tokyo, Japan) with 3 min of cooling on ice between rounds. The total RNA was extracted from the homogenate using a TRI reagent (Sigma-Aldrich, St.
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The residual DNA was eliminated by treating it with DNase I (Takara Bio, Shiga, Japan) during extraction and purification of the total RNA.

**Microarray experiment**

The Agilent Maize Oligo Microarray 4 × 44 K slides (Design ID 016047, Agilent Technologies, Santa Clara, CA, USA) was custom-designed with 42,034 in-situ synthesized 60-mer oligonucleotide reporters (Ma et al., 2008; Coetzer et al., 2011). It was used to process the gene expression profiles of a cDNA fragment for a transcript from the coleoptiles and from the mesocotyls of etiolated maize seedlings. Complementary RNA was synthesized with 200 ng of the total RNA sample, then the Cyanine 3 (Cy3)-labeled RNA was synthesized with Cy3-CTP using the Low Input Quick Amp Labeling Kit (Agilent Technologies) for microarray analysis according to the manufacturer’s protocol. The Cy3-labeled RNA was purified, fragmented, and then hybridized onto the microarray slides according to the manufacturer’s instructions. After hybridization at 65°C for 17 hours, the microarray slides were washed and scanned using the SureScan Microarray Scanner System (Model; G4900DA, Agilent Technologies). The degrees of hybridization signal, or intensity of the fluorescence, on the microarray slides were calculated using Feature Extraction Software (Agilent Technologies). Microarray analysis was conducted on three independent RNA samples obtained from etiolated maize seedlings grown in three independent maize boxes. Microarray data were analyzed using Gene Spring GX ver. 14.9.1 software (Tomy Digital Biology, Tokyo, Japan) and the maize microarray annotation database (Coetzer et al., 2011). Data were produced by normalizing to 75 percentile shift protocol and the baseline-to-median control sample protocol. Arrays were filtered on expression 20-100th percentile in the raw data. The P value was corrected by the False Discovery Rate (FDR) method. The resulting microarray data in this article can be found in the DNA Data Bank of Japan (DDBJ) BioProject database under DRA Accession number PRJDB11348.

**Results and Discussion**

For the study of gravity in the regulation of plant growth and development, space experiments often come with more novel challenges than do terrestrial ones. Etiolated maize (Zea mays L. cv. Golden Cross Bantam) seedlings grown under μg conditions on the ISS in space showed automorphogenesis, and a more enhanced PAT in shoots than those grown on Earth (Miyamoto et al., 2016; Oka et al., 2020). Nearly the same results were obtained in our previous BRIC-AUX experiment on the STS-95 mission (Ueda et al., 1999, 2000).

It should be mentioned that several comprehensive analyses using microarray techniques to analyze gravity-dependent alterations of gene expression have been applied to a number of biological species (Kimbrough et al., 2004; Lebsack et al., 2010; Fengler et al., 2015; Wakabayashi et al., 2015, 2017; Higashibata et al., 2016; Johnson et al., 2017). To identify genes that might be responsible for gravity-specific growth and development of etiolated maize seedlings, we compared gene expression profiles of shoots grown 1 g conditions on Earth and μg conditions on the ISS using the Agilent 016047 Maize 4 × 44 K microarray with 42,034 in-situ synthesized 60-mer oligonucleotide reporters representing approximately 80% of maize coding genes (Ma et al., 2008; Coetzer et al., 2011). Gene array data were analyzed according to stringent criteria that restricted the scored genes for specific hybridization values to at least twice the control level using software made by Coetzer et al. (2011). As a result, more than 32,000 transcripts (ca 75% of probe sets) were detectable in four microarray hybridizations with maize cRNA (Table 1).

**Transcriptional changes in etiolated maize seedlings grown under μg conditions on the ISS**

A volcano plot of gene expression is shown in Fig. 1. Red and blue points in the volcano plot represent genes whose expression was more than twice as high as the control level and with a statistical significance of \(P < 0.05\).

Analysis of the raw datasets revealed that 1030 transcripts accumulated at least a two-fold difference within the coleoptiles of etiolated maize seedlings grown under μg conditions on the ISS and 1 g conditions on Earth, 877 up-regulated and 153 down-regulated transcripts, and 428 up-regulated and 162 down-regulated transcripts in the coleoptiles and in the mesocotyls, respectively (Table 2A). Genes responsive to gravity might be more present in the coleoptiles than in the mesocotyls.

On the other hand, analysis of the raw datasets revealed that 5219 transcripts accumulated at least a two-fold difference in the coleoptiles than in the mesocotyls of etiolated maize seedlings grown on Earth (Table 2B). Of 5219 transcripts, 2171 and 3048 transcripts were respectively up-regulated and down-regulated from the coleoptiles compared with the mesocotyls under 1 g conditions on Earth. Under μg conditions on the ISS, 4559 transcripts were accumulated differently in the mesocotyls of etiolated maize seedlings, and 2116 and 2443 transcripts were up-regulated and down-regulated in the coleoptiles compared with the mesocotyls. These results suggest that gene expression is quite different in the coleoptiles from the mesocotyls.

**Table 1.** The number of transcripts in etiolated maize seedlings detected by the Agilent Maize 4×44 K microarray (Design ID 016047) with 42,034 probe sets to detect transcripts from 80% of the maize coding genes, filtered to expression of 20-100th percentile in the raw data. *Values in parentheses are percentages of transcripts in etiolated maize to the total number of probe sets.*

| Conditions   | Organs or gravity        | Number of transcripts detected |
|--------------|--------------------------|-------------------------------|
| μg vs 1 g    | Coleoptile               | 32152 (76.5%)                 |
| μg vs 1 g    | Mesocotyl                | 32344 (76.9%)                 |
| Coleoptile vs Mesocotyl | μg            | 32564 (77.5%)                 |
| Coleoptile vs Mesocotyl | 1 g           | 32616 (77.6%)                 |
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Venn diagram analysis of data sets of μg conditions on the ISS and 1 g conditions on Earth

Venn diagrams were constructed to identify the transcripts responsive to μg conditions in etiolated maize seedlings regardless of the differences between the coleoptiles and the mesocotyls (Fig. 2A). Transcripts of 10 and 154 genes were found to decrease and to increase, respectively, under μg condition in space compared with 1 g conditions (Table 3). This indicates that the number of genes commonly up-regulated under μg conditions in space was greater than those that were down-regulated. Although the total numbers of 154 and 10 were detected as commonly up-regulated and down-regulated genes there, respectively, regardless of organ difference, most of the functions of genes commonly up- and down-regulated under μg conditions in space regardless of the source organ were not clarified (Tables 3A and 3B).

Phytohormone-related genes, TC309752 (auxin-response factor 3) and TC283935 (gibberellin-2-beta-dioxygenase) were included (Tables 3A and 3B).

Venn diagrams also showed transcripts expressed in the coleoptiles and the mesocotyls regardless of gravity conditions (Fig. 2B). Of the transcripts, 1973 and 1411 transcripts commonly decreased and increased in the coleoptiles compared with the mesocotyls, respectively, regardless of gravity conditions, which suggests that the gene expression pattern of the coleoptiles is quite different from that of the mesocotyls of etiolated maize seedlings irrespective of gravity conditions.

Functional classification of genes up- and down-regulated in the coleoptiles and the mesocotyls of etiolated maize seedlings under μg conditions on the ISS

Figures 3 and 4 show the functional classification of genes up- and down-regulated under μg conditions in space with the respective organ. In the coleoptiles, 877 and 153 genes were up-regulated and down-regulated, respectively, under μg conditions in space, and 65% were unknown or not annotated (Fig. 3). Among up-regulated and down-regulated genes, several phytohormone-related genes were found to be up- or down-regulated in the coleoptiles. Several genes relating to the function of gene expression including signal transduction were also found to respond to gravity.

In the mesocotyls, 40-45% were unknown or not annotated in the genes up-regulated and down-regulated under μg conditions in space (Fig. 4). Similar to the

Table 2. Number of transcripts obtained by analysis of the effect of gravity conditions on gene expression in the coleoptiles and the mesocotyls of etiolated maize seedlings by microarray analysis with the Agilent-016047 Maize 4×44 K microarray

| Organ    | With a difference greater than 2 fold | Highly expressed under μg conditions in space | Weakly expressed under μg conditions in space |
|----------|---------------------------------------|---------------------------------------------|---------------------------------------------|
| Coleoptile | 1030                                  | 877                                         | 153                                         |
| Mesocotyl | 590                                   | 428                                         | 162                                         |

A

| Gravity conditions | With a difference greater than 2 fold | Highly expressed in the coleoptile [coleoptile]/[mesocotyl] | Weakly expressed in the coleoptile [coleoptile]/[mesocotyl] |
|-------------------|---------------------------------------|------------------------------------------------------------|------------------------------------------------------------|
| 1 g conditions    | 5219                                  | 2171                                                      | 3048                                                      |
| μg conditions     | 4559                                  | 2116                                                      | 2443                                                      |

B

Venn diagram analysis of data sets of μg conditions on the ISS and 1 g conditions on Earth

Venn diagrams were constructed to identify the transcripts responsive to μg conditions in etiolated maize seedlings regardless of the differences between the coleoptiles and the mesocotyls (Fig. 2A). Transcripts of 10 and 154 genes were found to decrease and to increase, respectively, under μg condition in space compared with 1 g conditions (Table 3). This indicates that the number of genes commonly up-regulated under μg conditions in
coleoptiles, several phytohormone-related genes were substantially found to be up- and down-regulated under μg conditions in the mesocotyls.

**Phytohormone-related genes and water channel genes up- or down-regulated in etiolated maize seedlings grown under μg conditions on the ISS**

We have already reported that six auxin-related genes – Auxin-induced protein SNG4, Indole-3-acetic acid amidase synthetase GH3.3, Auxin-induced protein, SAUR-like auxin-responsive family protein and Auxin response factor – were regulated by gravity using data from microarrays of Medicago (Medicago truncatula) and etiolated Alaska pea seedlings grown under μg and artificial 1g conditions on the ISS (Kamada et al., 2020). In the coleoptiles and the mesocotyls of etiolated maize seedlings grown under μg conditions in space, PAT has been reported to be greater than grown under 1g conditions on Earth (Miyamoto et al., 2019). Together with the fact that several auxin-related genes and water-channel genes are responsive to gravity in etiolated pea seedlings as mentioned above (Miyamoto et al., 2019; Kamada et al., 2020; Oka et al., 2020), phytohormone-related genes and water channel genes were focused in the raw datasets in etiolated maize seedlings. Tables 4 and 5 list phytohormone-related genes that are differently expressed under μg condition in space in the coleoptiles and the mesocotyls. Signal ratios calculated with the expression ratio greater than two are included.

In the coleoptiles of etiolated maize seedlings grown under μg conditions in space, 17 and 1 were up-regulated and down-regulated, respectively, as phytohormone-related genes (Table 4). It should be noted that expression of several auxin-related genes was up- and down-regulated under μg conditions in space (TC309752 encoding Auxin Response Factor 8, TC303498 encoding auxin responsive protein, LOC100284645 encoding SAUR family protein, and LOC100304432 encoding auxin conjugate hydrolase, and LOC100285511 encoding indole-3-acetate beta-glucosyltransferase).

On the other hand, in the mesocotyls, nine genes related to phytohormones and one gene related to water channel aquaporin were responsive to μg conditions in space (Table 5). It should also be noted that expression of several auxin-related genes were up-regulated (TC309752 encoding Auxin Response Factor 8, TC290939 encoding IAA25 [an auxin-responsive Aux/IAA family member], LOC10030406 encoding SAUR 33 [auxin-responsive SAUR family member], and LOC100191847 encoding an auxin-induced protein); several were down-regulated (LOC100279643 encoding a SAUR family protein, TC301840 encoding Aux/IAA protein, and CD947244 encoding auxin-induced-related) under μg conditions in space. These results strengthen support for the commonly accepted idea that the plant growth and development is substantially regulated by the network of phytohormones either under 1 g conditions on Earth or under μg conditions in space.

Phytohormones are considered to act as chemical mediators in response to environmental stimuli and endogenous physiological signals. Many studies of gene
Microarray analysis with maize seedlings grown in space

Table 3. Commonly expressed genes that change under μg conditions in space regardless of organ difference (the coleoptiles and the mesocotyls). The signal ratios calculated with an expression ratio >2 are included. Microarray data were analyzed using the Maize Microarray Annotation Database at http://MaizeArrayAnnot.bi.up.ac.za/ (Coetzer et al., 2011).

**A: Commonly up-regulated genes under μg conditions in space regardless of organ difference (the coleoptiles and the mesocotyls)**

| Probe name | Gene symbol | Putative annotation |
|------------|-------------|---------------------|
| A_92_P003827 | TC309752 | auxin response factor expressed |
| A_92_P040165 | TC253935 | gibberellin 2-beta-dioxygenase [Zea mays] |
| A_92_P06218 | TC301109 | amino acid permease [Zea mays] |
| A_92_P027068 | BG317220 | ATP-binding cassette, sub-family A (ABC1), member 9, isoform CRA_b [Homo sapiens] |
| A_92_P011423 | LOC100284433 | ZIM motif family protein [Zea mays] |
| A_92_P027282 | TC301163 | ZIM motif family protein [Zea mays] |
| A_92_P038612 | BM380732 | ZIM motif family protein [Zea mays] |
| A_92_P038838 | LOC100286212 | ZIM motif family protein [Zea mays] |
| A_92_P029595 | LOC100281370 | WRKY DNA binding domain containing protein [Zea mays] |
| A_92_P032910 | AC205562.3_FG002 | WRKY69-superfamily of TFs having WRKY and zinc finger domains [Zea mays] |
| A_92_P033858 | DBF4 | DRE-binding protein 4 [Zea mays] |
| A_92_P024914 | dBf5 | DRE-binding protein 3 [Zea mays] |
| A_92_P017026 | pco133091 | stress-induced transcription factor NAC1 [Oryza sativa Indica Group] |
| A_92_P017408 | pco133091 | stress-induced transcription factor NAC1 [Oryza sativa Indica Group] |
| A_92_P032015 | TC313829 | GM15425 [Drosophila sechellia] |
| A_92_P027167 | TC295487 | helix-loop-helix DNA-binding domain containing protein [Zea mays] |
| A_92_P007074 | TC281776 | HSI-like protein [Saccharum hybrid cultivar R570] |
| A_92_P029846 | CD991724 | HSI-like protein [Saccharum hybrid cultivar R570] |
| A_92_P028814 | 47.21266 | long cell-linked locus protein [Zea mays] |
| A_92_P00139 | TC288823 | phi-1-like phosphatase-induced protein [Zea mays] |
| A_92_P01352 | LOC100280482 | polcalcin Jun o.2 [Zea mays] |
| A_92_P014864 | TC303349 | PREDICTED: LOW QUALITY PROTEIN: RNA-binding protein 33-like [Ailuropoda melanoleuca] |
| A_92_P035916 | TC302807 | Ser/Thr receptor-like kinase, putative, expressed [Triticum aestivum] |
| A_92_P040191 | TC283019 | syntaxin 121 [Zea mays] |
| A_92_P026239 | TC297790 | type I phosphodiesterase/nucleotide pyrophosphatase family protein [Desulfovibrio magneticus RS-1] |

Others: 81 genes and 8 genes belonged to hypothetical proteins; some proteins of unidentified function; 20 genes were not annotated; 20 genes were unknown.

**B: Commonly down-regulated genes under μg conditions in space regardless of organ difference, the coleoptiles and the mesocotyls**

| Probe name | Gene symbol | Putative annotation |
|------------|-------------|---------------------|
| A_92_P031495 | TC300430 | LOC100284300 [Zea mays] |
| A_92_P011780 | CG773924 | hypothetical protein [Zea mays] |
| A_92_P01463 | CN944556 | hypothetical protein [Zea mays] |
| A_92_P029251 | LOC10030437 | hypothetical protein [Zea mays] |
| A_92_P001479 | MAGI4_38589 | hypothetical protein [Zea mays] |
| A_92_P017161 | CF384436 | predicted protein [Nematostella vectensis] |
| A_92_P003472 | DR909913 | NA |
| A_92_P023100 | BM800760 | unknown [Zea mays] |
| A_92_P028768 | LOC100382036 | unknown [Zea mays] |
| A_92_P039242 | LOC100382036 | unknown [Zea mays] |

expression using microarrays have been conducted on different plant species. As shown in Tables 4 and 5, the phytohormone-related transcript levels of 17 up-regulated and 1 down-regulated in the coleoptiles, and 9 up-regulated and 6 down-regulated in the mesocotyls of etiolated maize seedlings grown under μg conditions in space were recognized as compared with 1 g conditions on Earth. Semi-quantitative reverse transcriptase PCR and RNA blot analyses using total RNA prepared from independently isolated should be examined in the near future. Obtained results with the microarray analysis of phytohormone-related genes will provide improvement of our understanding of how plant growth and development and other physiological events respond to the changes in gravity.

Up- and down-regulated phytohormone-related and water channel related genes in the coleoptiles compare with the mesocotyls of etiolated maize seedlings grown under 1 g conditions on Earth, and of those grown under μg conditions in space were shown in Tables 6 and 7, respectively. Obtained microarray profiles will provide improvement of our understanding of differences in morphology and growth in response to gravity conditions in these organs.

Based on the data from microarray analysis, the mechanisms by which PAT was enhanced in etiolated maize coleoptiles grown in μg conditions in space were not clarified, although, as described above, several auxin-
related genes were substantially up- or down-regulated in μg conditions in space. Microgravity dramatically altered cellular localization of ZmPIN1a, an auxin efflux carrier in plasma membranes in parenchymatous cells of the coleoptiles, shifting mainly toward the vascular bundle. However, gene expression of ZmPIN1a and ZmAUX1 encoding auxin influx carrier did not increase in space (Oka et al., 2020). In this microarray analysis, μg
conditions in space had no significant effect on ZmPIN1a and ZmAUX1 gene expression. The expression levels of OsPIN1a and OsPIN1b, and OsAUX1 in rice seedlings detected by a microarray have been reported to be unaffected by μg conditions in space (Wakabayashi et al., 2017). These data support our previous suggestion that the mechanism of μg-enhanced PAT in maize shoots is more likely to be due to the enhanced ZmPIN1a accumulation and altered ZmPIN1a localization in the parenchymatous cells of the coleoptiles (Oka et al., 2020), although the mechanisms that enable the cells to acclimate to a different gravity are still poorly understood.

The expression of several water channel genes, Nodulin26-like intrinsic protein3-1, Plasma membrane

**Table 4.** Up- and down-regulated genes related with phytohormones in the coleoptiles of etiolated maize seedlings under μg conditions in space. The signal ratios calculated with the expression ratio >2 are included. FC indicates the multiple. Microarray data were analyzed using the Maize Microarray Annotation Database at http://MaizeArrayAnnot.bi.up.ac.za/ (Coetzer et al., 2011).

| A | Up-regulated genes in the coleoptiles under μg conditions in space compared with those under 1 g conditions on Earth |
|---|---|
| Probe name | Gene symbol | Putative annotation | FC |
| A_92_P003827 | TC309752 | auxin response factor 8 | 3.40 |
| A_92_P041727 | TC303498 | auxin responsive protein | 12.06 |
| A_92_P007680 | LOC100284645 | saur family protein | 3.73 |
| A_92_P035877 | LOC100304432 | auxin conjugate hydrolase | 9.20 |
| A_92_P040165 | TC283935 | gibberellin 2-oxidase | 2.61 |
| A_92_P004075 | DT652030 | tpa: gid1-like gibberellin receptor | 2.21 |
| A_92_P04220 | CO449604 | tpa: gid1-like gibberellin receptor | 2.17 |
| A_92_P014620 | TC288228 | tpa: gid1-like gibberellin receptor | 2.13 |
| A_92_P019259 | tps1 | ent-kaurene synthase b | 9.65 |
| A_92_P041713 | BM501430 | ent-kaurene synthase b | 5.76 |
| A_92_P025735 | DR970388 | cytokinin-O-glucosyltransferase 2 | 2.91 |
| A_92_P039216 | TC289805 | 1-aminoacyclopropane-1-carboxylate synthase | 2.80 |
| A_92_P021355 | LOC100285687 | ethylene-responsive transcription factor 2 | 3.48 |
| A_92_P026370 | LOC100274396/LOC100283499 | ethylene-responsive transcription factor 2 | 2.59 |
| A_92_P017575 | TC309475 | ethylene responsive element binding factor 5 | 2.24 |
| A_92_P040282 | LOC100278463 | ethylene-responsive transcription factor 4 | 4.32 |
| A_92_P035403 | TC288322 | 12-oxophytodienoic acid reductase | 3.75 |

| B | Down-regulated gene in the coleoptile under μg conditions in space compared with that under 1 g conditions on Earth |
|---|---|
| Probe name | Gene symbol | Putative annotation | FC |
| A_92_P019391 | LOC100285511 | indole-3-acetate beta-glucosyltransferase | 2.71 |

**Table 5.** Up- and down-regulated genes related to phytohormones and water channel in the mesocotyls of etiolated maize seedlings under μg conditions in space. The signal ratios calculated with the expression ratio >2 are included. FC indicates the multiple. Microarray data were analyzed using the Maize Microarray Annotation Database at http://MaizeArrayAnnot.bi.up.ac.za/ (Coetzer et al., 2011).

| A | Up-regulated genes in the mesocotyls under μg conditions in space compared with those under 1 g conditions on Earth |
|---|---|
| Probe name | Gene symbol | Putative annotation | FC |
| A_92_P003827 | TC309752 | auxin response factor 8 | 3.29 |
| A_92_P004637 | TC290939 | iaa25-auxin-responsive aux iaa family member | 2.19 |
| A_92_P039969 | LOC100304064 | saur33-auxin-responsive saur family member | 2.04 |
| A_92_P029268 | LOC100191847 | auxin-induced protein | 5.12 |
| A_92_P024419 | TC308787 | auxin response factor | 3.14 |
| A_92_P040165 | TC283935 | gibberellin 2-oxidase | 4.11 |
| A_92_P000187 | geranylgeranyl diphosphate synthase | 2.70 |
| A_92_P017575 | TC309475 | ethylene responsive element binding factor 5 | 2.17 |
| A_92_P040282 | LOC100278463 | ethylene-responsive transcription factor 4 | 3.05 |
| A_92_P020898 | NIP2-1 | nod26-like major intrinsic protein | 2.05 |

| B | Down-regulated genes in the mesocotyls under μg conditions in space compared with those under 1 g conditions on Earth |
|---|---|
| Probe name | Gene symbol | Putative annotation | FC |
| A_92_P016049 | LOC100279643 | saur family protein | 2.45 |
| A_92_P035458 | TC301840 | aux iaa protein | 2.09 |
| A_92_P012851 | CD947244 | auxin-induced-related, indole-3-acetic acid induced-related-like | 2.28 |
| A_92_P007812 | AC203966.5_FG005 | gibberellin 20 oxidase | 2.04 |
| A_92_P005300 | TC302601 | iso-kaurene synthase | 2.16 |
| A_92_P008699 | TC290707 | kaurene synthase2 [Zea mays] | 3.27 |
Table 6. Up- and down-regulated phytohormone-related and water channel-related genes in the coleoptiles compared with the mesocotyls of etiolated maize seedlings grown under 1 g conditions on Earth. The signal ratios calculated with an expression ratio >2 are included. FC indicates the multiple. Microarray data were analyzed using the Maize Microarray Annotation Database at http://MaizeArrayAnnot.bi.up.ac.za/ (Coetzer et al., 2011).

A: Up-regulated genes in the coleoptiles compared with the mesocotyls under 1 g conditions on Earth

| Probe name     | Gene symbol | Putative annotation                      | FC   |
|----------------|-------------|------------------------------------------|------|
| A_92_P008134   | LOC100274564| auxin response factor expressed           | 2.03 |
| A_92_P038369   | TC289436    | auxin response                            | 3.14 |
| A_92_P028948   | aic1        | auxin influx carrier component            | 2.22 |
| A_92_P033688   | TC300002    | auxin influx carrier component            | 2.58 |
| A_92_P031877   | TC280561    | auxin response factor expressed           | 3.49 |
| A_92_P031674   | DT53630     | auxin responsive protein                  | 3.51 |
| A_92_P029981   | TC312303    | iaa9-auxin-responsive aux iaa family member | 2.04 |
| A_92_P024977   | c12749_1    | auxin induced protein                     | 3.94 |
| A_92_P015153   | TC280560    | auxin response factor expressed           | 2.42 |
| A_92_P021173   | LOC100273544| auxin response factor expressed           | 2.84 |
| A_92_P037233   | LOC100279375| iaa16-auxin-responsive aux iaa family member | 2.46 |
| A_92_P018244   | LOC100283409| auxin response factor 75                  | 2.11 |
| A_92_P029268   | LOC100191847| auxin-induced protein                     | 3.20 |
| A_92_P027796   | LOC100273280| dormancy auxin associated expressed       | 2.56 |
| A_92_P035359   | TC285732    | dormancy auxin associated expressed       | 3.21 |
| A_92_P000534   | PIN10a      | auxin efflux carrier                      | 2.04 |
| A_92_P036865   | PIN10a      | auxin transport protein                   | 2.07 |
| A_92_P034419   | TC306787    | auxin response factor expressed           | 5.23 |
| A_92_P019180   | TC312866    | auxilin-like protein                      | 2.23 |
| A_92_P041021   | bx1         | indole synthase                           | 3.13 |
| A_92_P015991   | LOC100281876| gibberellin 2-oxidase                     | 3.31 |
| A_92_P007195   | LOC100279730| gibberellin receptor gid12                | 2.08 |
| A_92_P041347   | TC283646    | gibberellin receptor gid12                | 2.86 |
| A_92_P011603   | TC279907    | gibberellin-regulated protein 1 precursor | 10.91|
| A_92_P015997   | TC296753    | gibberellin receptor gid12                | 2.61 |
| A_92_P040561   | AC148152.3_FG005 | 1-aminoacyclopropane-1-carboxylate oxidase | 2.03 |
| A_92_P005060   | LOC100191321| 1-aminoacyclopropane-1-carboxylate oxidase | 2.90 |
| A_92_P030869   | TC296640    | ethylene-responsive transcription factor 3 | 2.20 |
| A_92_P026522   | TC303789    | ethylene-overproduction protein 1         | 2.15 |
| A_92_P041843   | LOC100278000| cytokinin-O-glucosyltransferase 2         | 2.85 |
| A_92_P010694   | LOC100194073| cytokinin-N-glucosyltransferase 1         | 4.13 |
| A_92_P037496   | LOC100216698| cis-zeatin O-glucosyltransferase          | 3.11 |
| A_92_P023501   | TC304292    | cytokinin-O-glucosyltransferase 2         | 3.73 |
| A_92_P009352   | opr6        | 12-oxophytodienoic acid reductase         | 2.43 |

B: Down-regulated genes in the coleoptiles compared with the mesocotyls under 1 g conditions on Earth

| Probe name     | Gene symbol | Putative annotation                      | FC   |
|----------------|-------------|------------------------------------------|------|
| A_92_P016049   | LOC100279643| saur family protein (Small Auxin Up-Regulated genes) | 2.62 |
| A_92_P002869   | TC282777    | gh3 family protein (Auxin-responsive GH3 family protein) | 4.42 |
| A_92_P009793   | TC291934    | iaa16-auxin-responsive aux iaa family member | 4.17 |
| A_92_P017110   | TC313452    | iaa9-auxin-responsive aux iaa family member | 4.88 |
| A_92_P013043   | TC303963    | auxin efflux carrier component            | 2.04 |
| A_92_P041401   | DR815209    | auxin efflux carrier component            | 8.89 |
| A_92_P041727   | TC303498    | auxin responsive protein                  | 21.73|
| A_92_P039969   | LOC100304064| saur33-auxin-responsive saur family member | 2.05 |
| A_92_P011352   | umc1527     | iaa12-auxin-responsive aux iaa family member | 2.68 |
| A_92_P023616   | LOC100193444| iaa15-auxin-responsive aux iaa family member | 2.52 |
| A_92_P031752   | CO446587    | iaa15-auxin-responsive aux iaa family member | 2.27 |
| A_92_P026164   | LOC100272577| iaa15-auxin-responsive aux iaa family member | 8.43 |
| A_92_P028798   | LOC100274580| iaa16-auxin-responsive aux iaa family member | 4.75 |
| A_92_P026777   | LOC100501604| saur33-auxin-responsive saur family member | 97.20|
| A_92_P035458   | TC301840    | aux iaa protein                           | 6.17 |
| A_92_P037187   | cs8808_2    | aux iaa protein                           | 10.59|
| A_92_P007353   | TC291881    | auxin-responsive family protein           | 3.73 |
| A_92_P014336   | LOC100384587| iaa14-auxin-responsive aux iaa family member | 3.75 |
| A_92_P001004   | LOC100281451| auxin efflux carrier family protein       | 4.52 |
| A_92_P015994   | DR970126    | auxin efflux carrier family protein       | 5.68 |
Microarray analysis with maize seedlings grown in space

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| Probe name | Gene symbol | Putative annotation | FC  |
|------------|-------------|---------------------|-----|
| A_92_P022309 | LOC100281432 | auxin-independent growth promoter | 2.79 |
| A_92_P034034 | LOC100281432 | auxin-independent growth promoter | 3.71 |
| A_92_P036263 | PIN5c | auxin efflux carrier component | 2.48 |
| A_92_P021795 | LOC100304260 | auxin-induced protein | 2.11 |
| A_92_P037345 | TC302307 | auxin conjugate hydrolase | 3.96 |
| A_92_P025204 | TC302430 | auxin-independent growth promoter | 4.51 |
| A_92_P005536 | LOC100501492 | auxin influx carrier component | 8.90 |
| A_92_P012851 | CD947244 | auxin-induced-related, indole-3-acetic acid induced-related-like | 2.00 |
| A_92_P026112 | TC299405 | auxin response factor 7a | 3.03 |
| A_92_P040141 | LOC100285200 | aux1-like permease | 2.26 |
| A_92_P007680 | LOC100284645 | saur family protein | 4.78 |
| A_92_P006412 | CD966839 | saur family protein | 2.61 |
| A_92_P006412 | NIP2-1 | nodulin-like protein 5ng4 | 12.34 |
| A_92_P036454 | LOC100279417 | nodulin-like protein 5ng4 | 10.02 |
| A_92_P028288 | TC290531 | indole-3-acetic acid-amido synthetase | 3.31 |
| A_92_P039996 | CF633343 | indole-3-acetic acid-induced protein | 26.73 |
| A_92_P035775 | LOC100283148 | gibberellin 2-oxidase | 5.16 |
| A_92_P033542 | LOC100285694 | gibberellin responsive1 [Zea mays] | 2.69 |
| A_92_P029216 | gar1 | ent-kaurene synthase-like protein 1 | 15.60 |
| A_92_P041504 | TC315348 | gibberellin receptor gid12 | 6.15 |
| A_92_P016737 | CD938481 | gibberellin 2-oxidase | 2.87 |
| A_92_P004220 | TC291345 | tpa: gid1-like gibberellin receptor | 2.15 |
| A_92_P014620 | CO449604 | tpa: gid1-like gibberellin receptor | 2.33 |
| A_92_P007812 | AC203966.5_FG005 | gibberellin 20 oxidase | 2.25 |
| A_92_P022237 | DR965584 | cytokinin-O-glucosyltransferase 1 | 4.37 |
| A_92_P025345 | CO530016 | cytokinin-N-glucosyltransferase 1 | 11.74 |
| A_92_P006993 | CO460823 | cytokinin-O-glucosyltransferase 3 | 4.86 |
| A_92_P039216 | TC298905 | 1-aminocyclopropane-1-carboxylate synthase | 13.01 |
| A_92_P017185 | TC310713 | 1-aminocyclopropane-1-carboxylate oxidase | 3.02 |
| A_92_P039018 | TC298909 | 1-aminocyclopropane-1-carboxylate synthase | 7.27 |
| A_92_P021355 | LOC100285687 | ethylene-responsive transcription factor 2 | 2.48 |
| A_92_P012448 | LOC100216872 | ethylene-responsive family | 2.70 |
| A_92_P024931 | DR816653 | ethylene-responsive family | 2.43 |
| A_92_P041084 | pco106446 | ethylene-responsive family protein | 2.30 |
| A_92_P026370 | LOC100274398/LOC100283499 | ethylene responsive protein | 4.58 |
| A_92_P034037 | TC289555 | ethylene-responsive protein | 3.23 |
| A_92_P027408 | TC315321 | ethylene-induced calmodulin-binding protein 4 | 2.18 |
| A_92_P040282 | LOC100278463 | ethylene-responsive transcription factor 4 | 2.66 |
| A_92_P013803 | BE643561 | ethylene response factor | 3.89 |
| A_92_P026121 | umc1393 | ethylene response factor | 2.73 |
| A_92_P001175 | TC307676 | 1-aminocyclopropane-1-carboxylate synthase | 5.30 |
| A_92_P024829 | TC283295 | brassinosteroid insensitive 1-associated receptor kinase 1 precursor | 2.79 |
| A_92_P014900 | TC293855 | brassinosteroid insensitive 1-associated receptor kinase 1 precursor | 2.64 |
| A_92_P030036 | CD651380 | jasmonate O-methyltransferase | 2.59 |
| A_92_P041718 | TC304534 | jasmonate O-methyltransferase | 3.33 |
| A_92_P033682 | NIP2-3 | nod26-like major intrinsic protein | 4.01 |
| A_92_P023471 | TC306588 | nod26-like major intrinsic protein | 8.07 |
| A_92_P06282 | pip2e | aquaporin | 3.27 |
| A_92_P05935 | TC311399 | aquaporin | 7.65 |
| A_92_P016372 | pip2c | plasma membrane intrinsic protein | 18.53 |
| A_92_P020820 | pip2d | plasma membrane intrinsic protein | 16.79 |
| A_92_P001145 | TC302043 | plasma membrane intrinsic protein | 138.07 |
| A_92_P031303 | tip2b | tonoplast intrinsic protein | 198.92 |
| A_92_P013456 | LOC100192638 | nod26-like major intrinsic protein | 4.40 |
| A_92_P020898 | NIP2-1 | nod26-like major intrinsic protein | 4.63 |

Intrinsic protein11, and AQUAPORIN1/Tonoplast intrinsic protein, were found to be gravity-regulated in etiolated pea seedlings (Kamada et al., 2020). NIP2-1 gene encoding nod26-like major intrinsic protein was up-regulated in etiolated maize seedlings grown under μg conditions in space (Table 5). These data also suggest that water channel genes function to maintain plant growth and development in space.

In the coleoptiles, 877 and 153 genes were up-regulated and down-regulated under μg conditions,
Table 7.  
Up- and down-regulated phytohormone-related and water channel-related genes in the coleoptiles compared with the mesocotyls of etiolated maize seedlings grown under μg conditions in space. The signal ratios calculated with the expression ration >2 are included. FC indicates the multiple. Microarray data were analyzed using the Maize Microarray Annotation Database at http://MaizeArrayAnnot.bi.up.ac.za/ (Coetzer et al., 2011).

A: Up-regulated genes in the coleoptiles compared with the mesocotyls under μg conditions in space

| Probe name     | Gene symbol | Putative annotation                           | FC   |
|----------------|-------------|-----------------------------------------------|------|
| A_92_P031877   | TC280561    | auxin response factor expressed               | 2.44 |
| A_92_P031674   | DT53630     | auxin responsive protein                      | 3.27 |
| A_92_P033267   | DR795294    | saur25-auxin-responsive saur family member    | 2.58 |
| A_92_P024977   | ci12749_1   | auxin induced protein                         | 4.29 |
| A_92_P020320   | CD995873    | iaa30-auxin-responsive aux iaa family member  | 3.48 |
| A_92_P021173   | LOC100273544| auxin response factor expressed               | 2.01 |
| A_92_P041021   | bx1         | indole synthase                               | 2.32 |
| A_92_P005060   | LOC100191321| 1-aminocyclopropane-1-carboxylate oxidase     | 3.33 |
| A_92_P041454   | LOC100191321| 1-aminocyclopropane-1-carboxylate oxidase     | 2.02 |
| A_92_P030869   | TC296640    | ethylene-responsive transcription factor 3    | 3.50 |
| A_92_P026522   | TC303789    | ethylene-overproduction protein 1             | 2.77 |
| A_92_P015991   | LOC100281876| gibberellin 2-oxidase                         | 2.76 |
| A_92_P007195   | LOC100279730| gibberellin receptor gid12                   | 2.51 |
| A_92_P032458   | TC284380    | cytokinin oxidase                             | 3.51 |
| A_92_P010694   | LOC100194073| cytokinin-N-glucosyltransferase 1            | 3.39 |
| A_92_P037496   | LOC100216698| cis-zeatin O-glucosyltransferase              | 2.20 |
| A_92_P003470   | LOC100502319| aquaporin                                     | 2.59 |

B: Down-regulated genes in the coleoptiles compared with the mesocotyls under μg conditions in space

| Probe name     | Gene symbol | Putative annotation                           | FC   |
|----------------|-------------|-----------------------------------------------|------|
| A_92_P001004   | LOC100281451| auxin efflux carrier family protein           | 2.84 |
| A_92_P015994   | DR970126    | auxin efflux carrier family protein           | 2.80 |
| A_92_P031666   | TC280891    | ettin protein                                 | 2.98 |
| A_92_P040141   | LOC100285200| aux1-like permease                            | 2.02 |
| A_92_P022309   | LOC100281432| auxin-independent growth promoter             | 2.80 |
| A_92_P034034   | LOC100281432| auxin-independent growth promoter             | 3.43 |
| A_92_P020346   | LOC100286028| iaa14-auxin-responsive aux iaa family member  | 2.32 |
| A_92_P009793   | TC291934    | iaa16-auxin-responsive aux iaa family member  | 8.37 |
| A_92_P017110   | TC313452    | iaa9-auxin-responsive aux iaa family member   | 4.80 |
| A_92_P013043   | TC303963    | auxin efflux carrier component                | 2.49 |
| A_92_P041401   | DR815209    | auxin efflux carrier component                | 6.16 |
| A_92_P039969   | LOC100304064| saur33-auxin-responsive saur family member    | 2.99 |
| A_92_P011352   | umc1527     | iaa12-auxin-responsive aux iaa family member  | 3.13 |
| A_92_P023616   | LOC100193444| iaa15-auxin-responsive aux iaa family member  | 2.94 |
| A_92_P031752   | CO446587    | iaa15-auxin-responsive aux iaa family member  | 2.48 |
| A_92_P026164   | LOC10027577 | iaa15-auxin-responsive aux iaa family member  | 6.14 |
| A_92_P036263   | PIN5c       | auxin efflux carrier component                | 2.95 |
| A_92_P028798   | LOC100274580| iaa16-auxin-responsive aux iaa family member  | 3.37 |
| A_92_P037345   | TC302307    | auxin conjugate hydrolase                    | 3.35 |
| A_92_P025204   | TC302430    | auxin-independent growth promoter             | 3.09 |
| A_92_P026777   | LOC100501604| saur33-auxin-responsive saur family member    | 68.64|
| A_92_P005536   | LOC100501492| auxin influx carrier component                | 6.55 |
| A_92_P035458   | TC301840    | aux iaa protein                               | 2.86 |
| A_92_P037187   | ci8808_2    | aux iaa protein                               | 4.21 |
| A_92_P007353   | TC291881    | auxin-responsive family protein               | 2.74 |
| A_92_P006797   | LOC100283322| iaa12-auxin-responsive aux iaa family member  | 2.03 |
| A_92_P014336   | LOC100384587| iaa14-auxin-responsive aux iaa family member  | 3.62 |
| A_92_P026112   | TC299405    | auxin response factor 7a                     | 2.47 |
| A_92_P028988   | TC290531    | indole-3-acetic acid-amido synthetase         | 4.06 |
| A_92_P010997   | TC304613    | indole-3-acetic acid inducible 31             | 11.53|
| A_92_P039996   | CF633343    | indole-3-acetic acid-induced protein          | 18.18|
| A_92_P016013   | CX129610    | indole-3-acetate beta-glucosyltransferase     | 3.76 |
| A_92_P024463   | LOC100274322| indole-3-acetate beta-glucosyltransferase     | 4.90 |
| A_92_P000591   | LOC100285511| indole-3-acetate beta-glucosyltransferase     | 2.31 |
| A_92_P035775   | LOC100285694| gibberellin 2-oxidase                         | 2.56 |
| A_92_P041504   | CD936481    | gibberellin receptor gid12                   | 4.70 |
| A_92_P016737   | TC291345    | gibberellin 2-oxidase                         | 2.26 |
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respectively. It should be mentioned that several genes related to cell wall metabolism were up-regulated, these encoding cellulose synthase (BM378963: FC 2.93; AW256077: FC 2.32), xyloglucan endoglycosylase (LOC10019158: FC 2.03), β-glucanase (TC280477: FC 2.32; LOC100384311: FC 2.71), and pectinacetylesterase family protein (TC305059: FC 2.15). In the mesocotyls of 428 and 162 genes up-regulated and down-regulated under μg conditions in space, several genes related to cell wall metabolism were up-regulated, these encoding pectin methylesterase (CO452292: FC 3.41), pectate lyase (CD651380: FC 2.41), and β-glucanase (LOC100384311: FC 2.71), and pectinacetylesterase family protein (TC305059: FC 2.15). In the mesocotyls of 428 and 162 genes up-regulated and down-regulated under μg conditions in space, several genes related to cell wall metabolism were up-regulated, these encoding pectin methylesterase (CO452292: FC 3.41), pectate lyase (CD651380: FC 2.41), and β-glucanase (LOC100384311: FC 2.71), and pectinacetylesterase family protein (TC305059: FC 2.15). A glycomics study of the BRIC-16-Cyt sample in Arabidopsis suggests that biosynthesis of xylan and pectic components of the cell might be impacted by μg conditions in space and cause a compositional difference in the cell wall matrix (Johnson et al., 2017). Microarray analysis as determined by the BRIC-16-Cyt microarray also revealed that several genes involved in cell wall modification were up-regulated in spaceflight: arabinogalactan proteins AGP31 (AT1G28290) and the xyloglucan XTH9 (AT4G03210) (Johnson et al., 2017). Maize seedlings grown under μg conditions in space showed automorphogenesis, the coleoptiles slightly being curved, and the mesocotyls being curved at random in a growth chamber maize box (Miyamoto et al., 2019). As already reported (Sugimoto et al., 2014; Johnson et al., 2017; Choi et al., 2019; Barker et al., 2020), it was not excluded the possibility that genes affected by μg conditions in space were related to redox control and/or stress responses in different plant organs as well. Together with the fact that several cell wall-related genes were up-regulated in maize seedlings, μg conditions in space led to structural differences in cell walls, possibly resulting in the changes due to morphogenesis.

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Declaration of Interests

The authors declare that there are no competing interests.
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