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ABSTRACT
Plants represent an essential part of future life support systems that will enable human space travel to distant planets and their colonization. Therefore, insights into changes and adaptations of plants in microgravity are of great importance. Despite considerable efforts, we still know very little about how plants respond to microgravity environments on the molecular level, partly due to a lack of sufficient hardware and flight opportunities. The plant Arabidopsis thaliana, the subject of this study, represents a well-studied model organism in gravitational biology, particularly for the analysis of transcriptional and metabolic changes. To overcome the limitations of previous plant hardware that often led to secondary effects and to allow for the extraction not only of RNA but also of phytohormones and proteins, we developed a new experimental platform, called ARABIDOMICS, for exposure and fixation under altered gravity conditions. Arabidopsis seedlings were exposed to hypergravity during launch and microgravity during the free-fall period of the MAPHEUS 5 sounding rocket. Seedlings were chemically fixed inflight at defined time points, and RNA and phytohormones were subsequently analyzed in the laboratory. RNA and phytohormones extracted from the fixed biological samples were of excellent quality. Changes in the phytohormone content of jasmonate, auxin, and several cytokinins were observed in response to hypergravity and microgravity.

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INTRODUCTION
Sounding rockets, such as the MAPHEUS rocket of the German Aerospace Center (DLR), provide up to 7 min of microgravity, and retrieval of the rockets within 1–2 h facilitates downstream sample analysis without the risk of sample degradation. MAPHEUS provides a platform for material physics and gravitational biology experiments in weightlessness. Flight opportunities are offered in an annual time frame. The ARABIDOMICS fixation unit was developed to fix large amounts of plant materials (up to 10 000 seedlings per experiment) on microgravity platforms such as drop towers, parabolic plane flights, and sounding rockets. The requirements of such a fixation unit were generated based on the experiences with the demands of modern high-throughput omics experiments and already existing fixation units used in microgravity. Mainly, the usage in drop towers and sounding rockets poses tremendous technical challenges. The fixation has to be automated, and the fixation time should be within a range of less than 2 s to best utilize the time of microgravity in the drop tower. In the past, syringe-based fixation systems were operated by hand. As an example, the Kennedy Space Center fixation tubes with three levels of containment were developed to allow astronauts to apply a fixative to
biological samples without the use of a glovebox. However, these fixation systems did not render them suitable for our desired range of platforms. Actuator-operated fixation systems for sounding rockets do not meet the demands for drop towers in terms of fixation time, as filling the experiment chambers takes longer than the time of microgravity in a drop tower. Existing hardware in the ISS, such as BRIC (Biological Research In Canisters), equipped with LED lights for plant experiments, can accommodate Petri Dish Fixation Units (PDFUs). However, plants grown on Petri dishes can cause drought stress in roots, or if roots grow into the medium, this makes it challenging to fix plants on time as the fixatives have to diffuse into the medium. An exquisite dry-ice-based freezing system was recently designed and constructed. Plant tissue was frozen in a steel tube where dry ice was created from carbon dioxide out of two gas cylinders conducted over a pressure system. The system was successfully tested on Arabidopsis seedlings during a parabolic plane flight. Time until shock freezing was reduced to under a minute. However, the rather fast fixation would suffice for sounding rockets but not for drop tower experiments. In addition, safety concerns would arise from the usage of gas cylinders, and expansion of the gas might cause turbulences during the sounding rocket flight. In summary, no existing plant fixation hardware is readily applicable to multiple platforms and would allow for harvesting of root and shoot materials.

For the work presented here, we made use of the MAPHEUS 5 flight opportunity to study immediate adaptation responses of biological systems (here plants) to altered gravity conditions on the level of transcript and phytohormone changes. Experiments within 7 min of real microgravity are part of an integrative approach to combine Arabidopsis transcriptome, proteome, and metabolome analyses in responses to short and prolonged reduced gravity treatments, which will finally be set together like a puzzle for the understanding of gravity signaling in plants. Short and longer microgravity treatments can best be accomplished by combining results from different microgravity platforms. The time needed for payload development and integration of individual low-gravity platforms can be very long: drop towers (~6 months), parabolic plane flights (~6 months), sounding rockets (~1–2 years), and even much longer for ISS experiments due to safety restrictions and limited access. We, therefore, developed a single hardware that can be operated on multiple platforms, does not require manual operation, and allows for fixation in the low second range. The result of this work is the ARABIDOMICS hardware (Fig. 1).

The focus of the subsequent molecular analysis of the Arabidopsis samples was on the level of RNA and phytohormones. Three phytohormones, ethylene, auxin, and jasmonates (JA), are known to play a role in the signaling pathways, leading to a gravitational response in plants. In this work, we focused on the non-volatile phytohormones auxin and JA.

Auxin gradients that determine the direction of cell growth and plant morphogenesis during, e.g., gravitropism, are established by polar auxin transport (PAT). Cholodny and Went independently reported that an asymmetry of auxin concentration in the plant organ leads to growth in a defined direction. The Cholodny–Went hypothesis has recently been expanded by discovering a jasmonic acid gradient in roots experiencing an altered gravitational vector, opposing and probably enhancing the auxin gradient.

In the long-term, an intricate understanding of the molecular responses in plants in response to microgravity will allow the modification of plants by breeding or genetic engineering in such a way that they can germinate, grow, and reproduce in space with optimum efficiency in microgravity/altered gravity conditions. Experiments on sounding rockets and thus within 6 min of microgravity provide an essential step to reach this goal. As a sounding rocket experiment is accompanied by acceleration (hypergravity) during launch before achieving free fall and thus microgravity conditions, the ARABIDOMICS hardware provides the possibility to fix plant samples at dedicated times in order to separate the effects induced by the different acceleration conditions.

**EXPERIMENTAL PROCEDURES**

**Plant material**

*Arabidopsis thaliana* seedlings of the ecotype Col-0 were grown hydroponically on stainless steel meshes (45 mm × 7 mm × 0.5 mm, mesh width 340 μm) on a reservoir filled with water for seven days before the start of the sounding rocket experiment. The reservoir was placed in an incubator set to 22 °C and a 16 h light/8 h dark cycle. Seedlings were integrated into the hardware 6 h before launch.

**Sample chambers**

The chambers were constructed in a manner to be vacuum-tight since the payload leaves the atmosphere during a sounding rocket flight, therefore being exposed to a few minutes of vacuum. To seal the chambers, o-ring sealings were used. An additional pressure vessel was not required due to the vacuum-tight setup of each sample chamber. Polycarbonate was chosen as the chamber-material due to its biocompatibility, low weight, and transparency, ideally suited for the cultivation of plants or other small kinds of organisms. Furthermore, observation and analysis of flooding and fixative distribution in the sample chambers under microgravity conditions are possible. Overall, 160 stainless steel meshes were integrated into 20 vacuum-tight sample chambers “Arablades” [Fig. 2(a)], 10 for hyper-g control plants and 10 for microgravity-treated plants. Arablades were integrated into the MAPHEUS 5 sounding rocket...
FIG. 2. ARABIDOMICS hardware (a) sample chamber (Arablade) made of polycarbonate with stainless steel nets for carrying young plant seedlings. Up to eight nets offer a total growth area of 2520 mm$^2$. (b) Fixation unit with a sample chamber, consisting of a syringe with preloaded spring, sample chamber, and magnetic valve to release the fixative into the sample chamber. (c) Late access unit containing 20 fixation units—10 units on each side. (d) Position of the temperature sensors (S1–S6) and the hypergravity and microgravity samples.

Phytohormone measurements

For phytohormone measurements, samples from multiple independent Arablades were pooled. Jasmonate and auxin analysis were conducted as described. In brief, ~10 mg of dried plant samples were extracted twice with MeOH:H$_2$O:HCOOH 15:4:1 (v/v/v), and the extracts were passed through a reversed-phase (RP) solid-phase extraction (SPE) column (96-well HR-X column, MACHEREY-NAGEL, 96 × 25 mg, catalog number: 738530.025M) to remove hydrophobic constituents. After evaporation of the MeOH and reconstitution with 1N HCOOH in H$_2$O, the samples were loaded onto a mixed-mode (RP and cation exchange) SPE column (96-well HR-XC column, MACHEREY-NAGEL, 96 × 25 mg, catalog number: 738540.025M). After washing the column with 1N HCOOH in H$_2$O, auxin and jasmonates were eluted from the column with 0.2 N HCOOH in 80% MeOH. For auxin analysis, the samples were concentrated by evaporation under an N$_2$-stream and subsequent reconstitution in a smaller amount of the same buffer. Chromatography was performed on a BRUKER Advance UHPLC system equipped with a Zorbax Eclipse XDB-C18 column (3 × 50 mm, 1.8 μm, Agilent Technologies) using gradient elution mode with 0.05% (v/v) HCOOH, 0.1% (v/v) ACN in H$_2$O as solvent A and in MeOH as solvent B. The chromatography was coupled to a Bruker Elite EvoQ Triple quad-MS equipped with a HESI (heated electrospray ionization) ion source. The instrument was operated in the multi-reaction-monitoring (MRM) mode. Chromatographic conditions (elution profile), ion-source parameter, and MRM settings were used as described in Ref. 15. For post-run analysis, we used the “MS Data Review” software of the “Bruker MS Workstation” (Version: 8.1.2). JA and JA-Ile were quantified based on identical isotopically labeled standards (D6-JA and D6-JA-Ile) that were added at the beginning of the extraction. COOH-JA-Ile was expressed relative to the D6-JA standard, and IAA was normalized to 4-methylumbelliferone, which was also added at the beginning of the extraction, since the signal of the isotopically labeled IAA (D5-IAA) was disturbed, probably by co-eluting constituents.

RNA extraction

Total RNA was extracted from 100 mg of frozen plant material with the RNaseasy Plant Mini Kit (QIAGEN). The quality of RNA was measured using an Agilent 2100 Bioanalyzer (Agilent Technologies). rRNA was removed with the Ribo-Zero Plant rRNA removal kit for plant leaves (Illumina). RNA libraries were prepared using the NEBNext Ultra Directional RNA Library Prep Kit (New England Biolabs). The cDNA library was run on the Illumina NextSeq 500 platform using the High Output 75 cycles kit at the Core Facility Genomics of the Medical Faculty Münster. Quality control of individual samples before alignment was performed using FastQC, and the results were summarized using MultiQC.
Operation schedule

Plant seedlings were grown in the laboratory, and the integration procedure of the seedlings inside the Arablade fixation chambers was started ~6 h before launch. All samples were handled in a horizontal position during the integration to avoid a gravity-dependent reaction. The late access to the experiment unit ARABIDOMICS was performed 90 min before launch. The first fixation was done after the hypergravity phase at t +68 s and after the microgravity phase at t +365 s. Ground controls were carried out in the ground support laboratory at the launch site at ESRANGE, Sweden.

RESULTS

ARABIDOMICS hardware development

ARABIDOMICS presents a system for the chemical fixation of seedlings under hypergravity and microgravity conditions. The aim was to investigate the gravity perception of plants of *Arabidopsis thaliana* during a 6-min parabolic flight aboard the MAPHEUS 5 sounding rocket. The design of the fixation unit was based on a late access system. This allows the whole preparation of the biological samples in the ground support laboratory and later the integration of the experiment unit inside the rocket structure. This avoids negative environmental influences of the rocket system inside the launcher.

The experiment ARABIDOMICS consists of three parts: the rocket structure with the experiment housing, batteries, communication electronics; a hatch to close the structure after the late access procedure; and the fixation unit, which is assembled from plant cultivation cuvettes and the Arablade chambers.

The outer structure was constructed according to the technical conditions for the rocket payload requirements. To protect the fixation unit and to prevent a critical influence of higher temperature from lift-off and re-entry, an aluminum housing was inserted in the rocket structure. Rails and fixation points, in combination with two fixation screws, secured the fixation unit inside the rocket structure. To fix biological samples in microgravity, special requirements are needed. The absence of gravity means that all liquids have to be pumped, and also air or bubbles in the hose system must be avoided to save the proper fixation with a liquid fixative. The task of fixing plants in an air-filled cuvette and the Arablade chambers.

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The late access unit integrates 20 individual fixation units and remains in the laboratory next to the launch site before the late access operations on the rocket, ~2 h before lift-off, start. The fixation system individually floods up to 20 sample chambers with methanol at desired time points, which chemically fixes the plants. Each sample chamber has its fixation unit, which makes the hardware very flexible in terms of the number of sample chambers per sample group and fixation times.

The bottom of the late access unit was built out of an 8 mm thick aluminum plate. On the bottom plate, an aluminum housing with two side walls and two end walls was attached, which serves to accommodate the 20 sample chambers integrated inside the fixation units. Quick couplings are used to connect the sample chamber with the fixation units.

Evaluation of hardware functionality

The ARABIDOMICS hardware was put to a verification test during a sounding rocket flight in the MAPHEUS 5 campaign on June 30, 2015, from the Ersrange Space Center in Sweden. The accelerations at the start of MAPHEUS 5 reached a maximum value of 11 g [Fig. 3(a)]. The blue “aRoll” curve describes the accelerations opposing the direction of flight generated by the two rocket stages. The red “aPitch” curve describes the acceleration generated by the rotation of the rocket. After burnout, the rocket rotates at about 2.7 Hz, which produces a centrifugal acceleration of about 3 g.

During the acceleration of the rocket, the inner walls of the modules reached a temperature of up to 120 °C. One critical parameter during the flight was, therefore, the temperature of the Arabidopsis seedlings. The evaluation of temperature data recorded by the ARABIDOMICS experiment during the MAPHEUS 5 flight showed that the sample chamber temperatures remained in the permissive temperature range of 17–25 °C [Fig. 3(b)]. Upon the release
of methanol, first for the hypergravity control (sensors 4 + 5) and later for the microgravity samples (sensors 1 + 3), the temperature in the sample chambers dropped down to 8–10°C.

**Differential hormone accumulation in seedlings exposed to sequential hypergravity and microgravity**

A hormone profile of Arabidopsis seedlings was recorded in response to sequential hypergravity and microgravity during a sounding rocket flight. Seedlings were fixed with methanol 68 s after launch, at the start of the microgravity phase, and 365 s after launch, 5 min into the microgravity phase [Fig. 3(a)]. As a ground 1 g control, a new batch of plants was fixed 2 days later on the ground in the identical hardware.

The most prevalent change in phytohormone profiling was a 2-fold decrease in the JA content in both hypergravity and microgravity samples. Auxin levels, on the other hand, were reduced in response to hypergravity, but this reduction was partially alleviated in the subsequent microgravity phase. Moreover, several cytokinins were changed in their abundance. Trans-zeatin-riboside, cis-zeatin-riboside, and isopentenyl-adenosine were increased in abundance in the microgravity sample. Other cytokinins, such as isopentenyl-adenine and cis-zeatin-o-glucoside, were increased only in the microgravity samples.

**Analysis of RNA extracted from samples fixed during a sounding rocket flight in the ARABIDOMICS hardware**

Besides the analysis of phytohormones, another focus of the project was to test the hardware for the analysis of transcriptional changes in response to the different g-levels during a sounding rocket flight (Fig. 4).

RNA was extracted from methanol-fixed plant materials using a commercial system and was analyzed in an Agilent Bioanalyzer. The RNA integrity numbers (RINs) varied between 8.1 and 9.5, which means excellent values for plant samples that were kept at room temperature for several hours after the sounding rocket flight and...
considering that RNA extracted from plants tends to have lower RINs due to the presence of plastidic 5.8S rRNA.

RNA sequencing of the extracted RNAs was performed, and quality control of the reads was done using FastQC and MultiQC. All reads were 80 bp long, and the Phred quality score for each base was above 30. Furthermore, the GC content was comparable for all samples. In total, the quality of the RNA was excellent for high throughput downstream analyses.
DISCUSSION

We have presented results from the analysis of Arabidopsis seedlings maintained at various g-levels during the MAPHEUS 5 sounding rocket campaign. RNA extracted from the biological samples after the sounding rocket flight and fixation with methanol showed superior RNA integrity numbers. Subsequent RNA sequencing resulted in good values for the number of reads and the Phred score.

The fixation with methanol also allowed for the analysis of metabolite changes in response to different g-levels. In this work, we focused on the quantification of phytohormones. The observed changes in abundance for JA and auxin that were only slowly beginning to alleviate at the end of the microgravity phase indicate that the effect on the phytohormone content is due to the high start accelerations. This is in agreement with studies on rice plants on board of the ISS, where no differences in the JA content were measured between microgravity and on-board 1g control plants. These results highlight that proper on-board controls are necessary. This can be achieved, either in the form of an additional sampling point at the beginning of the microgravity phase, by using a 1-g reference centrifuge on board during the flight and by performing hyper-gravity studies on the ground.

Moreover, it was shown that soybean lipoxygenase-1 has increased catalytic efficiency at low gravity during parabolic flights and might, therefore, be a direct molecular target of gravity. Lipoxigenases are the first enzymes in JA biosynthesis, which might explain changes in the JA content. Further work will be necessary to study the mechanism of how gravity affects the JA content and to elucidate the role of JA and interplay with auxin during adaptation to different gravity levels.

A multi-platform for studying Arabidopsis growth and testing experimentation hardware, ARABIDOMICS, has been developed for a flight on the MAPHEUS 5 sounding rocket. The experiment hardware provides increased flexibility for experiment-specific adaptation, such as the number of experiment groups and the nature of the fixative. The multi-platform design allows for better comparability between different microgravity platforms, including drop tower, centrifuge, and parabolic plane flights.

In the course of the payload development phase, the ARABIDOMICS hardware was tested in parabolic flights in Bordeaux (France) and drops in the Bremen drop tower. The utilization of these platforms during the payload development phase for a sounding rocket experiment offered a significant advantage by addressing potential issues early on. The multi-platform approach also leads to overall reduced system costs.

The flexibility of the ARABIDOMICS hardware makes it ideal for an integrative approach to combine Arabidopsis transcriptome, proteome, and metabolome analyses in responses to seconds to minutes of reduced and increased gravity treatments using a variety of platforms. The results will contribute to the understanding of early steps of gravity signaling in plants.

AUTHOR’S CONTRIBUTIONS

J.H., M.G., L.K., and R.H. conceived and designed the hardware. M.G., L.K., O.S., and J.H. performed the sounding rocket experiment. M.S. performed the phytohormone extraction and analysis. M.B. performed RNA extraction and analyzed the RNA sequencing data. A.W. performed RNA sequencing. R.H., J.H., and M.B. wrote the manuscript, and all authors reviewed and edited the manuscript.

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