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ORIGINAL ARTICLE

Changes in Gene Expression of *Arabidopsis Thaliana* Cell Cultures Upon Exposure to Real and Simulated Partial-*g* Forces

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Abstract Cell cultures of the plant model organism *Arabidopsis thaliana* were exposed to partial-*g* forces during parabolic flight and clinostat experiments (0.16 *g*, 0.38 *g* and 0.5 *g* were tested). In order to investigate gravity-dependent alterations in gene expression, samples were metabolically quenched by the fixative RNA*later*® to stabilize nucleic acids and used for whole-genome microarray analysis. An attempt to identify the potential threshold acceleration for the gravity-dependent response showed that the smaller the experienced *g*-force, the greater was the susceptibility of the cell cultures. Compared to short-term μg during a parabolic flight, the number of differentially expressed genes under partial-*g* was lower. In addition, the effect on the alteration of amounts of transcripts decreased during partial-*g* parabolic flight due to the sequence of the different parabolas (0.38 *g*, 0.16 *g* and μg). A time-dependent analysis under simulated 0.5 *g* indicates that adaptation occurs within minutes. Differentially expressed genes (at least 2-fold up- or down-regulated in expression) under real flight conditions were to some extent identical with those affected by clinorotation. The highest number of homologuous genes was detected within seconds of exposure to 0.38 *g* (both flight and clinorotation). To a considerable part, these genes deal with cell wall properties. Additionally, responses specific for clinorotation were observed.

Keywords *Arabidopsis* · Cell cultures · Clinostat · Gene expression · Parabolic flight · Partial-*g* forces

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Introduction

The effect of different gravitational environments on the physiology, development and growth of plants has gained increasing attention, especially under the aspect of Moon and Mars missions (Stutte et al. 2006; Hasenstein 2009; Wyatt and Kiss 2013; Zupanska et al. 2013; Ruyters and Braun 2014; Ueda et al. 2014; Bizzarri et al. 2015). A range of experiment facilities exists to investigate effects of real microgravity (μg) such as airplanes, sounding rockets, satellites and space shuttles/station (Brinckmann 2005; Ruyters and Friedrich 2006; Astrium GmbH and Friedrichshafen 2012; Brinckmann 2012). In contrast, platform and thus investigations of partial-*g* forces upon plant

physiology are rather limited (for a review see Kiss 2014). Partial-g forces are defined to exist between μg during spaceflights and on planets with less mass than the Earth. The investigation of plant responses to partial-g (e.g. martian or lunar gravity) can provide us with important information about the possible use of plants within bio-regenerative life-support systems (Jones et al. 2012). On long-term missions to the Moon or to Mars, plants could recycle waste products and provide a less hostile environment for the crew. Experiments under partial-g can also help to identify the potential threshold acceleration that is required to induce a response due to reduced gravity. The threshold acceleration is one of the important parameter used to characterize the underlying processes of sensing gravity in plants (Volkmann and Sievers 1979).

In 1965, Gordon et al. first addressed the threshold of perception in plants in combination with acceleration and force vectors (Gordon and Shen-Miller 1965). In this context, first ground-based experiments were performed that exposed *Avena sativa* seedlings to partial-g forces. They demonstrated that significant growth responses within gravity-compensated seedlings can be associated with organ-specific threshold accelerations between 10^{-4} g for roots and 10^{-2} g for shoots on a centrifuge-clinostat (Shen-Miller et al. 1968). In primary cress roots, the threshold for the perception of mass accelerations was identified to be at 4.3×10^{-3} g, as investigated under simulated microgravity conditions using a fast-rotating clinostat (Sobick and Sievers 1979). The first experiment in space was performed with lettuce on board the Salyut-7 station. In contrast to other experiments, the threshold accelerations were calculated mathematically after the flight to be at 1.5×10^{-4} g for roots and 2.9×10^{-3} g for hypocotyls (Merkys et al. 1986). Similar to Sobick and Sievers (1979), the value of 2.6×10^{-3} g was confirmed for cress hypocotyls and roots a few years later (Laurinavicius et al. 1998).

The number of experiments that address the analysis of partial-g forces on different organisms is still very limited. Due to the access to the on-board centrifuges at the International Space Station or on sounding rockets, the number of experiments is increasing (Brinckmann 2005; Astrium GmbH and Friedrichshafen 2012). For example, Richter et al. showed that the threshold acceleration for the characteristical gravity-related movements in *Euglena gracilis* should be between 0.08 g and 0.12 g (Richter et al. 2001). Sounding rocket experiments with the alga *Chara* indicated that the displacement of statoliths occurred above 0.14 g of lateral acceleration (Limbach et al. 2005). For the root curvature of lentil seedlings, that were grown under μg before stimulation, a threshold acceleration of 1.4×10^{-5} g was estimated (Driss-Ecole et al. 2008). Unfortunately, data about the potential threshold acceleration for responses to altered gravitation are very limited for *Arabidopsis thaliana*. Kiss observed that the positive phototropic curvature upon blue and red light illumination is affected between 0.1 g and 0.3 g, however, no significant difference was detected between 0.3 g and 1-g (Kiss 2014). Plant cells not specialized in graviperception such as plant cell cultures exhibited different thresholds (Wolverton and Kiss 2009). Nevertheless, we have given considerable evidence that cell cultures of *Arabidopsis* are (1) sensitive to gravitational changes and (2) constitute a rather homogeneous test system which is easy to handle (Maier et al. 2003; Martzivanou and Hampp 2003; Babbick et al. 2005; Martzivanou et al. 2006; Babbick et al. 2007a; Babbick et al. 2007b; Barjaktarović et al. 2009; Neef et al. 2012; Fengler et al. 2013; Hausmann et al. 2013; Neef et al. 2013; Fengler et al. 2015) (see also Manzano et al. 2012; Zupanska et al. 2013). These cells respond to altered gravitational field strengths with changes in transcript levels, modulation of proteins, cellular redox state, as well as the concentration of different second messengers (Hausmann et al. 2013). Thus, we expected that partial-g forces can also be investigated in *Arabidopsis* cell cultures as well, as shown by Manzano et al. (2012). By simulating reduced gravitation via magnetic levitation, these authors could identify a number of common genes on the gene expression level for 0.1 g and simulated μg in contrast to 1-g.

In the present study, the generation of partial-g forces was achieved during parabolic flights (Joint European Partial-g Parabolic Flight, JEPPF) operated by the German Aerospace Center (DLR, Germany) together with CNRS (France) and Novespace (Bordeaux, France). Thereby, *Arabidopsis* cell cultures were exposed to partial-g forces, in addition to the regular hyper-g phases of a parabola (1.8 g). In parallel, cell cultures were exposed to simulated partial-g forces due to clinorotation (fast-rotating clinostat, compare Shen-Miller et al. 1968; Sobick and Sievers 1979).

By means of microarray data we could provide further evidence for the susceptibility of *Arabidopsis* cell cultures to partial-g forces. We hypothesize that, compared to μg (10^{-2} to 10^{-3} g during parabolic flights), cell cultures respond to partial-g forces to a different extent. In addition, we suppose that there is a potential threshold acceleration that is able to induce a response within the cell cultures. During parabolic flights, we could observe a decrease of the number of differentially altered transcripts owing to the sequence of the parabolas (0.38 g, 0.16 g, μg) This could also apply for clinorotation treatments with increasing time of exposure (minutes-range). In conclusion, there is still the question whether real and simulated conditions induce a similar response at the gene expression level, or if the cell cultures respond to the simulation *per se*.

Table 1 Summary of important experiment parameters including hardware, cultivation conditions, mode of operation, rotational speeds and fixation system that were used during partial-*g* parabolic flights and ground-based clinostat experiments

Experiment (abbreviation)	Hardware	Cultivation	Exposure time	*g*-level	Mode of operation	Fixation
Parabolic flight (PF)	Flight rack for cell cultures within glove box inside the Airbus A300	plastic flask	20 s 33 s 24 s 20 s	1.8 *g* 0.38 *g* 0.16 *g* 10^{-3} *g*	transiently alternating *g*-level	manual injection of RNA*later*®
Clinostat (C①)	fast-rotating 2-D clinostat	Petri dish	5, 10, 30 min	0.5 *g* 0.38 *g* 0.16 *g*	radius: 4.5 cm 3.4 cm 1.4 cm speed: 100 rpm	manual addition of liquid nitrogen
Clinostat (C②)	fast-rotating 2-D clinostat	Petri dish	33 s	0.38 *g*	radius: 3 cm 117 rpm	manual injection of RNA*later*®
Clinostat ([C②])	fast-rotating 2-D clinostat	Petri dish	22 s 33 s	1.8 *g* 0.38 *g*	radius: 3 cm 105 rpm (centrifugation) 117 rpm (clinorotation)	manual injection of RNA*later*®

Material and Methods

Cell Cultures

A suspension culture of wild type *Arabidopsis thaliana* (cv. Columbia) plants was generated from leaves and grown as described previously (Martzivanou and Hampp 2003; Fengler 2015). New medium was added each week. Seven days before an experiment, an aliquot of a six-day-old suspension culture (3 g) was spread on I.2a culture medium containing 1.6 % agar in either culture plastic flasks or Petri dishes (Greiner Bio-One, Frickenhausen, Germany).

After another 7 days of culture, the cells were exposed to partial-*g* conditions during parabolic flights, as well as to simulated reduced gravity (clinorotation). The differences between the different experiments are summarized in Table 1. After the experiments, the samples were metabolically quenched®, and prepared for whole genome microarray analysis.

Partial-*g* Forces During Parabolic Flight

The cell cultures were integrated into a special hardware as constructed for parabolic flights (Hausmann et al. 2013) (Fig. 1). Exposure was similar to μg parabolic flights (Airbus A300, Novespace, France). However, by adapting the flight angle and the over-all velocity of the plane, the cells were additionally exposed to 0.38 *g* and 0.16 *g* (Mars and Moon gravitation, Joint European Partial-*G* Parabolic Flight program). Compared to a μg parabola (about 20 s), the dura-

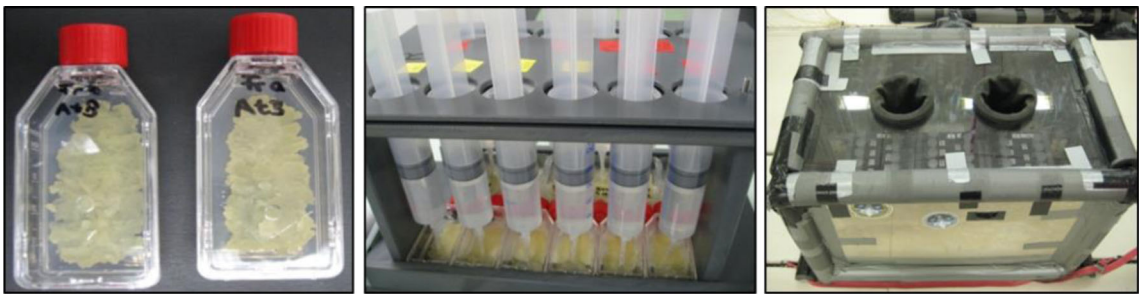

Fig. 1 *Arabidopsis* cell cultures, and flight hardware used during the partial-$g/\mu g$ parabolic flight campaign. *Left*: Six-day-old *Arabidopsis thaliana* (cv. Columbia) wild type cell cultures in cell culture plastic flask containing solid 1.2a medium (1.6 % agar). *Middle*: Flight rack with a syringe system containing the fixative RNA*later*® for metabolic quenching of samples. *Right*: Glove box attached to the plane, containing cell cultures and syringes

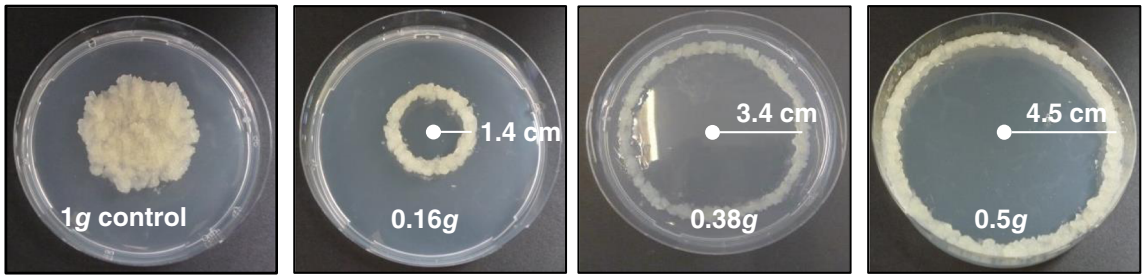

Fig. 2 *Arabidopsis thaliana* cell cultures during the first clinostat experiment (C①). Cell culture material was positioned in rings on Petri dishes with different radii (1.4, 3.4, 4.5 cm). The center of the dish is also the center of rotation. This resulted in the simulation of partial-*g* forces (0.16 *g*, 0.38 *g*, 0.5 *g*). Plates were vertically inserted and rotated at 100 rpm (fast-rotating 2-D clinostat, DLR, Cologne)

tion of a partial-*g* phase is longer (33 s for 0.38 *g*, 24 s for 0.16 *g*, see Table 1). The parabola sequence started with the highest *g*-force (0.38 *g*) during the first 13 parabolas, followed by 13 parabolas of 0.16 *g* and 5 μg parabolas.

Simulation of Partial-*g* Forces by Clinorotation

Two clinostat studies were performed in 2013 (C①) and 2014 (C②) in which we wanted to simulate partial-*g* forces. For this purpose, we used the fast-rotating 2-D clinostat with one rotation axis (DLR, Cologne, Germany). In both of the clinostat experiments the different parameters like the age of the cell culture and media composition were identical to those of the flight experiment (PF) described above.

In order to simulate different *g*-forces in the first experiment (C①), the cells were positioned in rings with different radii on the Petri dishes (1.4, 3.4, 4.5 cm; Fig. 2). By means of vertical rotation of the plates with 100 rpm simulated partial-*g* forces of 0.5 *g*, 0.38 *g* and 0.16 *g* were achieved ($a = \omega^2 * r$ with r = radius; ω = angular velocity; a = acceleration). The 1-*g* control plates were positioned inside the clinostat housing (not rotated), placed vertically. Cells cultures were exposed for 5, 10 and 30 min. In the second clinostat approach (C②), the cells were placed on a circle with a radius of 3 cm (Fig. 3). In this case, the mode of operation was optimized to be as close to parabolic flight conditions as possible. The profile of a parabola includes an initial hyper-*g* phase of 1.8 *g*. For that reason, the cells were centrifuged and experienced a 1.8 *g* phase for 22 s (similar to the PF) before they were exposed to clinorotation (this treatment is identified by [C②] in the following text). Hyper-*g* was achieved by centrifugation of the Petri dishes with 105 rpm with a laboratory centrifuge (MUSIC, Multi Sample Incubation Centrifuge, DLR). The Petri dishes were placed in a vertical position on the axis of the centrifuge rotor. During the parabolic flight the cells experienced 0.38 *g* during the first parabola. Therefore, we focused on the simulation of martian gravity (0.38 *g*) in C②, due to the possible adaptation of cells to altered gravity, along with the number of completed parabolas. This was obtained by vertical rotation with 117 rpm for 33 s (cells on a circle with a radius of 3 cm result in 0.38 *g*), immediately after centrifugation with 1.8 *g*.

Sample Fixation

For sample acquisition, the cell cultures were metabolically quenched at the end of the different gravity phases during the first parabola. This was achieved by manual injection of 30 ml RNA*later*® at 1-*g* before the first parabola, at the end of the initial hyper-*g* phase (1.8 *g*), at the end of the partial-*g* phase, and at the end of the second hyper-*g* phase.

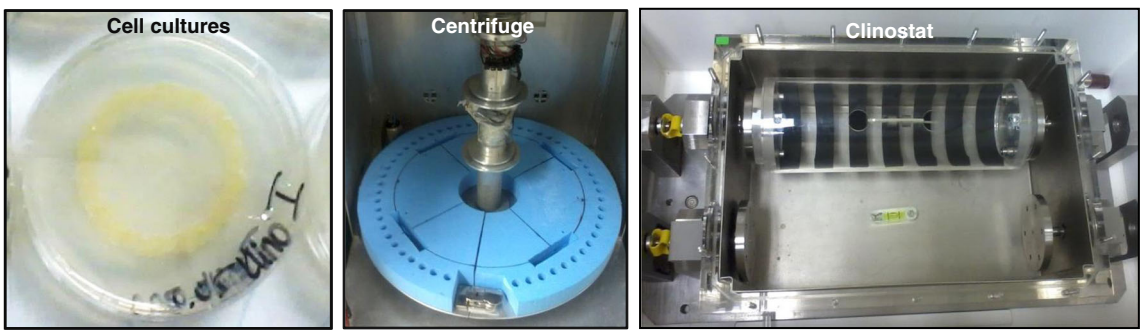

Fig. 3 *Arabidopsis thaliana* cell cultures during the second clinostat experiment (C②). Cell cultures were placed on Petri dishes in rings (radius 3 cm). The cell cultures were centrifuged with 1.8 *g* (105 rpm) in advance. Immediately afterwards, the plates were vertically inserted and rotated within the fast-rotating 2-D clinostat at 117 rpm, resulting in 0.38 *g* (both devices DLR, Cologne)

For the simulation experiment C①, the cultures were scraped off the agar immediately after exposure and fixed in liquid nitrogen. In C②, we tried to eliminate differences that might result from different fixation systems. Therefore, metabolic quenching of samples was carried out by manual addition of RNA*later*® (as in PF). After sample acquisition (all 3 experiments) the cells were incubated at −80 °C until further analysis.

Microarray Analysis

For microarray studies, total RNA was extracted, processed and hybridized with the Affymetrix GeneChip® (ATH1) as described previously (Fengler et al. 2013; Hausmann et al. 2013; Fengler et al. 2015). Expression data were calculated according to the detected signal intensity (raw values). For microarray analysis, we used the open-source Mayday Software (Battke et al. 2010). Normalization was performed with all datasets to achieve comparability and estimate $\log_2$ expression values as described (Bolstad et al. 2003; Irizarry et al. 2003a; Irizarry et al. 2003b; Fengler et al. 2015). The number of differentially expressed genes (DEGs) was determined by fold change calculation of $\log_2$ transformed expression data. Thereby, the threshold was set at $-1 \geq \log_2(\text{fold change}) \geq 1$ for at least 2-fold significantly altered transcripts (Mutch et al. 2002). To visualize relationships and correlations within the total number of expression values (nearly 23,000 values) due to similar experimental conditions, cluster analysis was performed within Mayday, and visualized as unrooted hierarchical clustering based on the clusters formed (Simonsen et al. 2008). In addition to Mayday, the R software package was used to calculate the Pearson correlation coefficient between the different treatments and illustrated as heat map with a colour code (Langfelder and Horvath 2008). In order to interpret functional ontologies, we used Gene Ontology terms provided within Mayday and by the Gene ontology consortium (Ashburner et al. 2000).

Results

This study summarizes gene expression data of *Arabidopsis* cell cultures which were exposed to real and simulated partial-*g* forces as experienced during parabolic flights and clinostat experiments. The resulting transcriptomes were analyzed in order to a) identify a potential threshold acceleration for responses in gravitational-related gene expression in *Arabidopsis* cell cultures, b) to investigate the suitability of clinorotation to simulate partial-*g* effects, and c) to screen for similarities between real and simulated partial gravity in gene expression.

Potential Threshold Above Martian Gravity

Cell cultures respond to 0.16 *g*, 0.38 *g* and 0.5 *g* during parabolic flights and ground-based experiments to a different extent (Fig 4).

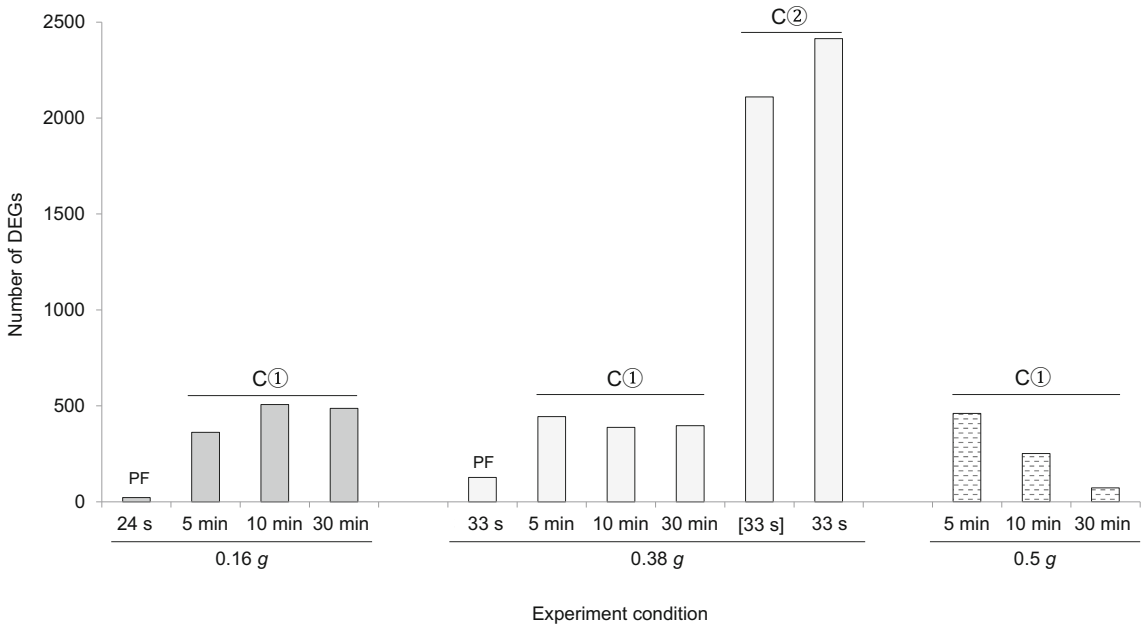

Fig. 4 *Arabidopsis thaliana* cell cultures were exposed to 0.16 *g* (coloured in *grey*), 0.38 *g* (*white*) and 0.5 *g* (*striped*) during parabolic flight (PF) and clinostat experiments (C① and C②) The number of differentially expressed genes (at least 2-fold up- or down-regulated) is displayed as a function of the time of exposure (seconds or minutes). The time in parantheses [33 s] represents the experiment with a preceding hyper-*g* treatment [C②]

Interestingly, the number of at least 2-fold differentially expressed genes (DEGs) under partial-g was much lower than under short-term μg (10^{-2} to 10^{-3} g) during a parabolic flight (thousands of DEGs during the first parabola, Neef et al. 2012; Hausmann et al. 2013). The sequence of parabolas was such that they started with 0.38 g, followed by 0.16 g, and ended with μg. The slightly higher response to 0.38 g in comparison to 0.16 g could thus be due to this sequence. At the first exposure to 0.16 g, the cells had already experienced twelve 0.38 g parabolas.

In the clinostat experiment C① there was no clear difference between the response to 0.16 g and 0.38 g, with no obvious time-dependent changes over 30 min. In contrast, simulated 0.5 g (not available under flight conditions) resulted in a clear decrease in DEGs from 5 to 30 min (Fig. 4). A simulation of the parabolic flight as shown under C② (33 s; with or without preceding 1.8 g) resulted in a considerably higher number of altered transcripts (Fig. 4). This finding suggests a decreasing number of altered transcripts with increasing time of exposure.

Effect of Simulated Partial-g Conditions on Gene Expression

The calculation of the Pearson's Correlation Coefficient (CorP) showed that the effect of the different experimental conditions on the whole transcriptome was relatively low (response of a small fraction of genes). Considering the total number of generated gene expression values (about 23,000), this resulted in a high CorP (Fig. 5). The clustering of expression values (again based on 23,000 genes) suggests that in the clinorotation experiment, gene expression was more affected by the time of exposure (cluster I to III, experiment C①), than by the change in gravity (0.16 g, 0.38 g or 0.5 g generate no separate cluster for a given time of exposure) (Fig. 6). The parabolic flight data form their own cluster (cluster IV, Fig. 6). Interestingly, under clinorotation, 30 min of exposure cluster with 1-g controls. This indicates that effects of clinorotation probably start to disappear after that time.

Experiment C② was focused on the simulation of 0.38 g. To be as close to parabolic flight conditions as possible we tested both, the effect of simulated 0.38 g for 33 s (clinorotation only), as well as the same treatment with a preceding 1.8 g phase which lasted for 22 s [C②] (compare Table 1). The response within the seconds range was more prominent than that upon prolonged time of exposure (Fig. 4). In C② nearly 2,500 genes (0.38 g simulation only) were differentially expressed (Fig. 4). The preceding hyper-g treatment ([C②]) reduced the number of DEGs (2,110 DEGs). In detail, 2,414 DEGs were differentially expressed under C② conditions (1,267 up- and 1,147 down-regulated, Fig. 7). Less (2,110) DEGs were altered with preceding hyper-g treatment [C②] (1,102 up- and 1,008 down-regulated, Fig. 7). The effects induced by both short-time simulation experiments were, however, very similar (C② and [C②]); 1,872 DEGs were identical (995 up-, 877 down-regulated, Fig. 7). A minor group of altered genes were specific for either treatment (C② 542 DEGs, [C②] 238 DEGs). With regard to the effect of the simulation, we were especially interested in genes affected by short-time clinorotation. These also comprise candidates which were identical under real and simulated conditions (44 and 68 DEGs; Fig. 7).

Simulated and Real Partial-g Forces: A Comparison

The clustering in Fig. 6 illustrates the low similarity between real (as experienced during PF) and simulated partial-g within the range of minutes. This is shown by the separation of clusters generated from parabolic flights (cluster IV) and clinostat samples (clusters I to III). In addition, the correlation showed a time-dependent decrease with the weakest correlation after 30 min (CorP 0.92) (Fig. 5). However, screening at the single gene level identified many similarities (named identical DEGs) between

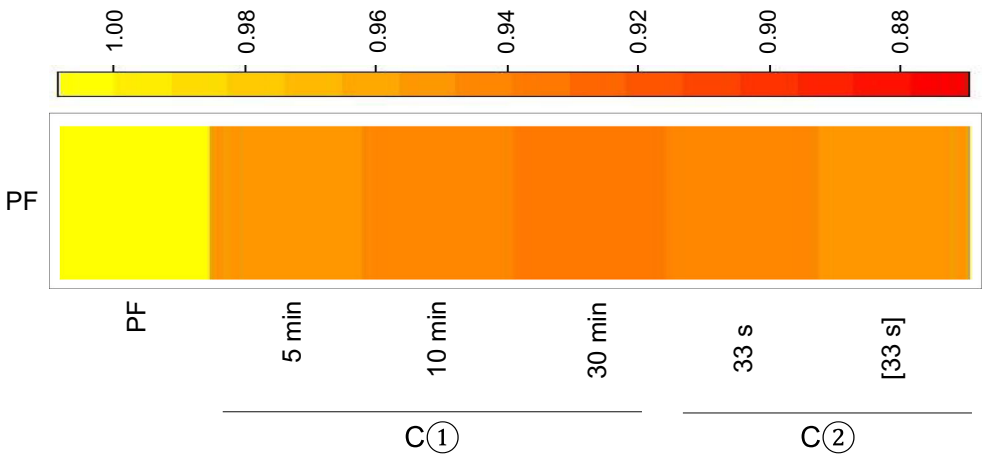

Fig. 5 Pearson correlation heat map. This shows the degree of similarity between real 0.38 g, as experienced during the partial-g parabolic flight (PF), and simulated conditions during the two clinostat experiments (C①, C②). The correlation value is related to the colour code above. The correlation is calculated on the large scale expression values (nearly 23,000 represented on the microarray)

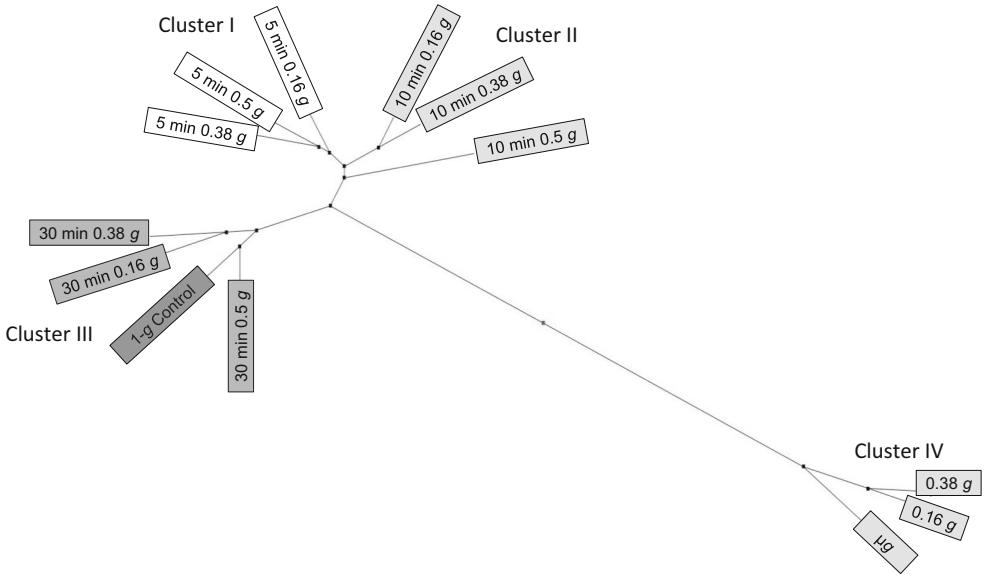

Fig. 6 Unrooted hierarchical clustering of gene expression data representing the response to partial-*g* forces experienced during parabolic flight (PF, cluster IV) and first clinostat experiment (C①, clusters I-III) based on the correlation between the different experiment conditions

real and simulated conditions. This was mainly the case for the seconds-range of exposure to 0.38 *g*. Out of 127 DEGs which responded during flight conditions (Fig. 4), 68 genes could also be induced under simulated conditions (54 %, C②). In case of the preceding hyper-*g* treatment, this number was reduced (44 identical genes). Among these genes (68 and 44) 43 were identical. These genes (overlapping square area in Fig. 7) are listed in Table 2. Among them were gene candidates that code for proteins that are known to be involved in the modification of cell wall properties like pectate lyase-like membrane proteins, glycosylhydrolases, a xyloglucanendotransglucosylase and proteins that are not characterized yet. Additionally, there were peroxidases, auxin-related proteins (e.g. PINs), transmembrane proteins and components of intracellular signaling chains like different protein kinases, transcription factors, and phytohormone-related proteins.

Discussion

Arabidopsis thaliana cell cultures are highly sensitive to a change in environmental conditions including gravity (Paul et al. 2005; Babbick et al. 2007a; Barjaktarović et al. 2009; Paul et al. 2011; Paul et al. 2012; Zupanska et al. 2013; Aubry-Hivet et al. 2014). We thus used this system to investigate short-term effects of reduced gravity on plant gene expression by whole genome microarrays. The

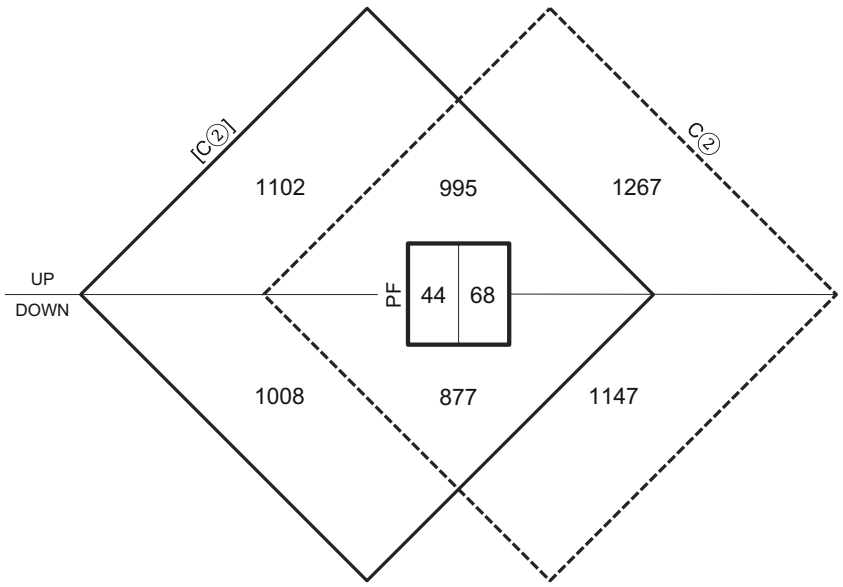

Fig. 7 Number of at least 2-fold differentially expressed genes (DEGs). The upper half (*UP*) shows up-, the lower half (*DOWN*) the number of down-regulated DEGs. The numbers in the peripheral area of squares give DEGs that respond to C② and [C②] (in total 1,102, 1,008 and 1,267 and 1,147). The overlapping area in the middle (*square*) shows DEGs identical between C② and [C②] (995 and 877). DEGs identified to be identical in all three experiments C②, [C②] and parabolic flight (PF) (44 and 68, not divided into up- and down-regulated ones) are highlighted in the overlapping centre of the figure

Table 2 List of identical genes that were at least 2-fold differentially expressed under 0.38 *g* during parabolic flight (PF) and simulated conditions during the clinostat experiment C② (without preceding hyper-g phase) and [C②] (with short-term hyper-*g*). Genes are represented by their gene identifier and gene name/description

No	ATG number	Gene name/description
1	AT1G03820	unknown protein
2	AT1G04680	pectin lyase-like superfamily protein
3	AT1G09450	haspin-related protein kinase
4	AT1G10970	zinc transmembrane transporter 4
5	AT1G22885	unknown protein
6	AT1G23080	PIN7 protein
7	AT1G29980	unknown membrane protein
8	AT1G30040	gibberellin 2-beta-dioxygenase
9	AT1G70940	PIN3 protein
10	AT1G71380	glycosyl hydrolase 9B3
11	AT1G75500	integral membrane protein
12	AT2G07751	NADH:ubiquinone/plastoquinone oxidoreductase
13	AT2G15830	unknown protein
14	AT2G32990	glycosyl hydrolase 9B8
15	AT2G40480	unknown protein
16	AT2G43520	trypsin inhibitor protein 2
17	AT2G47860	phototropic-responsive protein
18	AT3G08030	unknown protein
19	AT3G14205	phosphoinositide phosphatase protein
20	AT3G24503	aldehyde dehydrogenase
21	AT3G54260	trichome birefringence-like protein
22	AT3G56170	Ca^{2+}-dependent nuclease (CAN)
23	AT4G03210	xyloglucan endotransglucosylase (XTH) 9
24	AT4G14630	germin-like protein 9
25	AT4G15630	casp-like protein 1E1
26	AT4G21960	peroxidase 1
27	AT4G24780	pectate lyase protein
28	AT4G25200	heat shock protein 23.6
29	AT4G36110	small auxin-related protein
30	AT4G39320	microtubule-associated protein
31	AT5G05340	peroxidase 52
32	AT5G12340	unknown protein
33	AT5G13205	copia-like retrotransposon family
34	AT5G16250	unknown protein
35	AT5G22270	salt-induced/ein3/eil1-dependent protein
36	AT5G26220	chac-like protein
37	AT5G41810	unknown protein
38	AT5G46050	peptide transporter protein 3
39	AT5G46590	NAC-domain containing transcription factor
40	AT5G61480	leucine-rich repeat transmembrane kinase
41	ATCG00020	photosystem II reaction center protein a
42	ATCG00420	NADH dehydrogenase subunit protein
43	ATCG00430	NADH dehydrogenase subunit protein

generated gene expression data is considered to be typical for the initial steps in sensing partial-*g* forces, as shown for 0.38 *g* (Fig. 8).

In contrast to earlier studies were only parabolas yielding microgravity were flown (over 5,000 DEGs), the number of DEGs under parabolic flight partial-*g* was low during the first parabola (compare Hausmann et al. 2013). In the case of μg flights, we always only analyzed the first parabola of a given flight day, where the cells experienced microgravity for the first time. In the present study this was different. As the partial-*g* parabolas were flown in a sequence which started with 0.38 *g*, only the cells of this group experienced this reduced gravity for the first time. Those, exposed to 0.16 *g* for the first time had experienced 13 0.38 *g* parabolas before, those with μg (not shown), additional 13 0.16 *g* parabolas. This could explain the decreasing response. For the parabolic flights, we thus focus on the 0.38 *g* data in this study. The respective correlations between these and the clinorotation data are summarized in Figs. 5 and 7.

The Pearson correlations were generally quite good between real 0.38 *g* and the simulations (Fig. 5). This was not unexpected because this correlation includes the total number of 23,000 generated expression values of the array from which only a fraction responded with an at least two-fold change in transcript abundance (DEGs). Therefore, a high response in the second clinorotation experiment (many DEGs in the seconds-range compared to 1-*g* control) resulted in a low correlation (0.9 without preceding hyper-*g* phase, and even less with it: 0.88). The correlation was better in the range of minutes (0.98 for 5 min due to a weaker response). Nevertheless, we found a considerable range of regulated gene products that were identical under PF and simulated conditions (Table 2). One important overlap is an impact on cell wall organization. The majority of identical candidates (PF and C②) are involved in the modification of cell wall components, like pectate lyase-like membrane proteins (AT1G04680, AT4G24780; up-regulated). This probably decreases the mechanical strength of the primary cell wall by cleaving the α-1, 4-linked galacturonic acids which leads to a depolymerization of pectins (Sun and van Nocker 2010). Also up-regulated were the glycosyl hydrolases 9B3 (also named cellulase 3, AT1G71380) and 9B8 (AT2G32990), as well as a xyloglucane endotransglucosylase 9 (AT4G03210). These proteins are located in the plasma membrane or in the cell wall and are able to cleave the glycosidic bonds of polysaccharides. This can lead to cell wall expansion by polysaccharide degradation (Minic and Jouanin 2006). As shown for the simulation of reduced *g*-forces by magnetic levitation (Manzano et al. 2012), the expression of peroxidase genes (peroxidase 52 (AT5G05340), peroxidase 42 (AT4G21960)) is also up-regulated. Peroxidases are involved in the detoxification of reactive oxygen species in the extracellular matrix, which

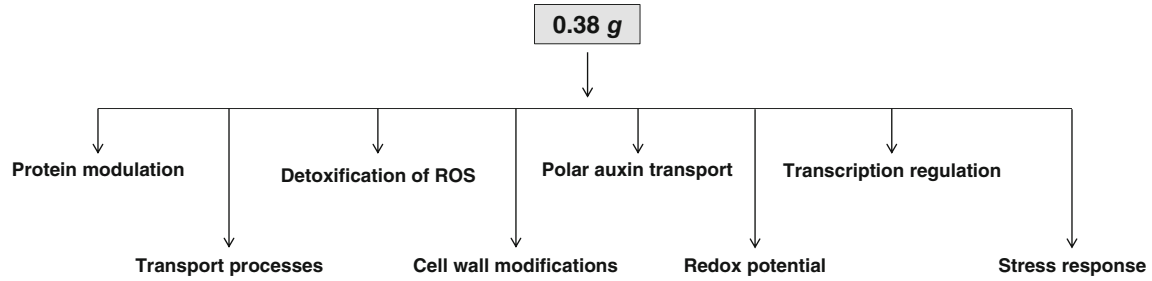

Fig. 8 Schematic overview of gene expression changes that are induced by 0.38 *g* (partial-*g*) in *Arabidopsis thaliana* cell cultures. Schematic presentation of gene expression changes is based on 68 differentially expressed genes that were identical under real (PF) and simulated 0.38 *g* (C②) conditions

always are part of stress responses. PIN3 (AT4G36110) and PIN7 (AT1G23080), two auxin-related proteins that are known to be sensitive to gravitational changes (Aubry-Hivet et al. 2014), as well as the small auxin-responsive protein (AT4G36110), are differentially expressed under both real and simulated 0.38 *g*. Furthermore, diverse integral membrane components appeared to be affected by both treatments, like the proteins of AT4G15630, AT1G75500 and different transmembrane peptide transporters (AT1G10970, AT5G46050). In addition, members of intracellular signaling pathways are sensitive to partial-*g* and changed in transcript abundance upon both treatments. These are e.g. the NAC-domain containing transcription regulator (ANAC096, AT5G46590) located in the nucleus, the haspin-related protein kinase (AT1G09450), an inositol phosphatase (AT3G14205) and a transmembrane protein kinase (AT5G61480). This leucine-rich repeat kinase is involved in phytohormone-related signaling pathways, like the cytoplasma-located gibberellin-2-beta-oxygenase (AT1G30040). Finally, the altered expression of reaction center proteins of the photosystems show that components of photosynthetic (ATCG00020, ATCG00420, ATCG00430) and the respiratory electron transport (NADH ubiquinone/plastoquinone oxidoreductase, AT2G07751) are affected by partial gravity levels as well. Other DEGs which respond to partial-*g* are not annotated to date (e.g. AT1G03820, AT1G22885, AT1G29980, AT2G15830, AT2G40480, AT3G08030, AT5G12340, AT5G16250, AT5G41810).

Processes induced especially under clinorotation, were transport, development, protein metabolism, signal transduction, or transcription. In addition, we found a considerable number of genes involved in the post-translational modification of proteins, such as diverse leucine-rich repeat membrane-bound kinases.

Owing to the sequence of parabolas, the partial-*g* parabolic flights can obviously not be used for the investigation of gravitational thresholds in *Arabidopsis*, due to the short and repetitive time of exposure to altered accelerations. Simulation by clinorotation of different gravitational field strengths revealed the least effects after 30 min at constant 0.5 *g*. After this period the number of DEGs was lowest. The unrooted hierarchical clustering (involves 23,000 expression values per sample) shows that this could also hold for 0.38 *g* and 0.16 *g* in the minutes-range (all 30 min treatments cluster with the 1-*g* control, Fig. 6). We thus assume that after this time of clinorotation specific responses are fading, demonstrating the insusceptibility of the cell cultures over time.

There is still the question what samples experience and perceive during fast clinorotation. In principal, the simulation of μg by means of the clinostat operates via randomization of the gravitational vector (Herranz et al. 2013) which will result in dependency of the sensitivity of the exposed systems – in omnilateral stimulation or a stimulus free environment (μg), similar as experienced during space flight (Klaus et al. 1998; Briegleb 2007; Herranz et al. 2013). The 68 DEGs which were detected to be identical between real and simulated conditions are out of 23,000 genes (0.6 %). This is not much but obviously specific for 0.38 *g*, because it is different from that induced upon μg in similar cell culture material (Hausmann et al. 2013; Fengler et al. 2015). As the majority of these DEGs is involved in the modification of cell wall molecules, the adaptation of cell wall properties to reduced gravity is a basic response (Hoson et al. 2002; Volkmann and Baluska 2006; Wang et al. 2006), and can obviously be simulated by clinorotation. Other homologies, such as detoxification of reactive oxygen species, are mostly stress responses which occur under a range of environmental alterations.

Taken together this study suggests that molecular responses to partial-*g* are in part different from those induced by μg, and that clinorotation can induce changes in levels of transcripts similar to flight experiments.

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