EVALUATION OF SIMULATED MICROGRAVITY ENVIRONMENTS INDUCED BY DIAMAGNETIC LEVITATION OF PLANT CELL SUSPENSION CULTURES

Khaled Y. Kamal1,8, Raúl Herranz1,8,*,
Jack J.W.A. van Loon2,3, Peter C.M. Christianen4, F. Javier Medina1,*

1Centro de Investigaciones Biológicas (CSIC), C/ Ramiro de Maeztu, 7 CP 28040 Madrid, SPAIN;
2European Space Research & Technology Center - TEC-MMG Lab. – European Space Agency (ESTEC-ESA), NETHERLANDS;
3Dutch Experiment Support Center (DESC) @ Dept Oral and Maxillofacial Surgery/Oral Pathology, VU University Medical Center / Dept Oral Function and Restorative Dentistry, Academic Centre for Dentistry Amsterdam (ACTA), Gustav Mahlerlaan 3004, NL-1081 LA Amsterdam, NETHERLANDS;
4High Field Magnet Laboratory (HFML), Institute for Molecules and Materials, Radboud University Nijmegen, NETHERLANDS;

*These authors have contributed equally to this work.
§Dr. Kamal present address is Faculty of Agriculture, Zagazig University, EGYPT.

*Corresponding authors: Dr. Raúl Herranz, Email: r.herranz@csic.es and Dr. F. Javier Medina, Email: fjmedina@cib.csic.es
Phone: +34 918373112 Ext. 4261 Fax: +34 915360432.

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**ABSTRACT**

Ground-Based Facilities (GBF) are essential tools to understand the physical and biological effects of the absence of gravity and they are necessary to prepare and complement space experiments. It has been shown previously that a real microgravity environment induces the dissociation of cell proliferation from cell growth in seedling root meristems, which are limited populations of proliferating cells. Plant cell cultures are large and homogeneous populations of proliferating cells, so that they are a convenient model to study the effects of altered gravity on cellular mechanisms regulating cell proliferation and associated cell growth. Cell suspension cultures of the *Arabidopsis thaliana* cell line MM2d were exposed to four altered gravity and magnetic field environments in a magnetic levitation facility for 3 hours, including two simulated microgravity and Mars-like gravity levels obtained with different magnetic field intensities. Samples were processed either by quick freezing, to be used in flow cytometry for cell cycle studies, or by chemical fixation for microscopy techniques to measure parameters of the nucleolus. Although the trend of the results was the same as those obtained in real microgravity on meristems (increased cell proliferation and decreased cell growth), we provide a technical discussion in the context of validation of proper conditions to achieve true cell levitation inside a levitating droplet. We conclude that the use of magnetic levitation as a simulated microgravity GBF for cell suspension cultures is not recommended.
Introduction

Plants on Earth are subjected to a constant gravitational field, which played a major role in their evolution (Hoson et al 1996, Ishii et al 1996). Gravity is the only environmental parameter which has remained constant on Earth since life appeared on the surface of our planet, regarding both the direction and magnitude of the gravity vector (Herranz & Medina 2014, Morita 2010). Therefore, evolution has provided a number of different solutions to meet the mechanical challenge of supporting the weight of a living organism (Raff 1996). In general, the influence of gravity on the physiology of an organism increases with its mass. In plants, gravity has an important effect on development by establishing the growth direction via the sedimentation of heavy components (statoliths) in specialized cells (Boonsirichai et al 2002, Herranz et al 2014, Kiss 2000), but gravitational effects have also been reported in cell cultures without specialized gravisensing organelles (Barjaktarović et al 2007, Kamal et al 2015, Manzano et al 2012, Martzivanou et al 2006).

Magnetism is another environmental factor affecting the life on Earth. The natural magnetic field strength at the surface of the Earth varies from 30 to 60 μT (Buffett 2010), but short-term exposures to high energy and high gradient magnetic fields of up to 13 T are being used regularly in diagnostic non-invasive techniques, such as magnetic resonance imaging, without any apparent long-lasting effects on cells (Weissleder et al 2000). Nevertheless, strong magnetic fields were reported to produce significant effects on the behavior and development of living organisms (Denegre et al 1998, Glover et al 2007, Maret & Dransfeld 1985), inhibition of Drosophila oogenesis (Herranz et al 2012a) or alteration of the Arabidopsis genome expression (Manzano et al 2012). In addition, the magnetic susceptibility of the materials at the molecular level is the basis of the phenomenon of diamagnetic levitation, which has been used to generate conditions of simulated microgravity in experiments with living beings. It is precisely the diamagnetic properties of the main component of cells (water) that it is experiencing a repelling magnetic force under a high energy and high gradient magnetic field (Beaugnon & Tournier 1991, Berry & Geim 1997). When using levitation as microgravity simulation, together with gravitational stress we can observe other effects induced by the high magnetic field, some of them heterogeneous, due to the different diamagnetic susceptibility of the various components of
the cell. The extent of these secondary effects should be confirmed in each experiment (Berry & Geim 1997, Herranz et al 2013).

The phenomenon of levitation has attracted wide attention due to its peculiarity and potential applications. Among various types of levitation methods, diamagnetic levitation is the most suitable for levitating large biological organisms (Berry & Geim 1997, Geim et al 1999). Placing a biological material in a high magnetic field with a strong magnetic field gradient creates a diamagnetic force on the system (Brooks et al 2000, Ueno & Iwasaka 1997). Orienting this force against the gravitational force leads to the absence of any net force on the object, thus, levitation occurs. Adjusting the polarity of the gradient field can vary the magnetic force. The effective gravity acting on a diamagnetic body in the magnetic field is defined in Eq. 1 as the net force, that is, the sum of the gravitational (m·g) and magnetic force ($F_m$), per unit of mass.

$$F_m = xVB \frac{dB}{dZ} \mu_0^{-1}$$

Eq.1

where: $x$ = Magnetic susceptibility, $V$ = Object Volume, $B$ = Magnetic field magnitude/flux density, $dB/dZ$=Magnetic Field Gradient and $\mu_0$= Permeability of the vacuum.

It should be remind that biological organisms are complex non-uniform materials and their levitation is due to the interaction of the magnetic field with all the molecular components of the subject, according to their average diamagnetic susceptibility. In fact, many of these molecular components have diamagnetic susceptibility of different magnitude. However, for biological materials in the magnetic field, it is useful to calculate the effective gravity acting on water, assuming that water (a diamagnetic molecule) is the major component of the living matter and that magnetic susceptibilities of all cells and tissues of the organism are similar to that of water (Berry & Geim 1997, Herranz et al 2013). A successful levitation of water droplets using magnetic levitation as a microgravity simulator has been demonstrated earlier (Beaugnon & Tournier 1991). Indeed, magnetic levitation of live frogs, grasshoppers, Arabidopsis seedlings and other biological systems has been demonstrated, using these criteria (Guevorkian & Valles 2004, Herranz et al 2012b, Hill et al 2012, Manzano et al 2013, Simon & Geim 2000, Valles et al 2005).

This paper reports and reviews the experience that we have obtained in experiments in which a magnetic levitation facility was used to provide a simulated microgravity environment in a plant.
suspension culture, in the context of a multinational GBF project (Herranz et al 2013). We have specifically analyzed the behavior of individual cells in suspension within a droplet of culture medium which was levitated under conditions corresponding to the levitation point of water. The applicability of this technology to gravitational studies on cell suspension cultures is discussed, as well as the secondary effects caused by the use of high energy and high gradient magnetic fields.

**Material and Methods**

1- Cultivation of fast growing *Arabidopsis* cell suspension cultures (MM2d)

*Arabidopsis thaliana* cell suspension culture MM2d (ecotype *Landsberg erecta*), was used (May & Leaver 1993). This culture remains under actively growing conditions while remaining in darkness. Consequently, the cells were grown in MSS medium (MS medium (Murashige and Skoog, Duchefa, The Netherlands) supplemented with 3% (w/v) sucrose, pH 5.8, adjusted with 1M NaOH) preserving darkness by covering the culture chambers with aluminum tape until the experiment insertion into the magnet bore. This medium was autoclaved at 110°C and stored at 4°C, then it was supplemented with 50 mg/l MS vitamins (Murashige and Skoog vitamin mixture, Duchefa), 0.5 mg/l NAA (α-naphthalene acetic acid, Duchefa) and 0.05 mg/l kinetin (Kinetin, Duchefa) sterilized by filtration using Minisart® filter unit. Faster growing derivatives of MM2d cells were selected uniquely by subculture in MSS medium (1:20 dilution every 7 days in 250 ml Pyrex flask), under shaking and darkness conditions (120 rpm in an Excella™ E24 shaker incubator, New Brunswick product by Eppendorf, USA) at 27°C (Menges & Murray 2006).

2- Diamagnetic Levitation Experiment at HFML

The magnetic levitation facility at the High Field Magnet Laboratory (HFML) is hosted by the Radboud University, Nijmegen (The Netherlands). It was used to observe simultaneously several altered gravity levels into the same high energy magnetic field environment of up to 16.26 T (Perenboom et al 2004, Wiegers et al 2010). We indicate the presence of high intensity magnetic fields in the samples by an asterisk (*). These conditions require a high power consumption of about 5.8 MW which results in a significant restriction on the total number of hours available for
our experiment (mainly during the night). In addition, the system needs to be cooled with a flow of 142 l/s of cold water at 12ºC (Christianen 2010) (Figure 1). Consequently, an upper limit to our experiment duration is set on 4-5 hours. This levitator has a central bore where the tubes (cut to be 26.5 mm height) are inserted at different distances from the center of the apparatus (maximum field), and consequently exposed to differential magnetic field and effective gravity conditions. This bitter system allowed us to obtain 4 different altered gravity and magnetic field samples (Figure 1D); A) Two simulated microgravity samples (0g* unstable, 0g* levitated) in which the magnetic field intensity is 13.46T (52.5mm form the center), and 10.35T respectively (78.75mm from the center), but the magnetic field gradient is maximized, producing a magnetic levitation force high enough to compensate the 1g force (calculated for pure water), required to generate two alternative simulated microgravity conditions. The first one is an unstable 0g* point and the second one is stabilized by the own magnetic field forcing the droplet to remain in the precise 0g* levitation point. B) Partial gravity (0.37g* Mars-like gravity) is generated by placing the sample at 26.25mm from the center (15.53T is the magnetic force intensity at that distance from the center of the magnetic bore). C) Internal control (1g*) was placed at the center of the apparatus, in which the more intense magnetic field, 16.26T, is present but it caused no diamagnetic force, since magnetic field gradient is null in this point (so the net 1g* gravity force is present).

Experiment duration was 3 hours. Three successive replicates were performed using Arabidopsis thaliana MM2d suspension cultures at confluence (7 days from last refreshing) in the magnetic levitation facility. 1 ml of the suspension culture was placed into 5 container tubes (4 localized in different distances from the center to generate different gravity levels (Figure 1D) plus one located outside of the magnet as 1g control) at RT (24ºC). All samples into the bore remained in darkness at any time as well as the 1g external control (covered with aluminum tape). After the end of each replicate, cells were recovered and distributed as samples to be either fixed or frozen, depending on the different analyses to be performed.
3- Flow cytometry and other Cell Cycle analyses

**Determination of DNA content of individual cells (% of cells in cell cycle phases)**

Frozen samples (500 mg cells pellet) were treated with the High Resolution Kit for plant ploidy level analysis (Kit Cystain UV precise P; type P containing solution A (Nuclei extraction buffer) and solution B (Nuclei staining buffer containing DAPI), Partec GmbH, Munster, Germany) (Menges & Murray 2006). Cells were carefully chopped with a sharp razor blade in 300 µl of solution A, and then incubated for 2 min at 4°C. Extracted nuclei solution was filtered using a nylon mesh (50µm), prior to adding 600 µl of solution B at 4°C in dark conditions. On average, 10000 particles were counted by flow cytometry approach (Cell sorter FACS Vantage, Becton-Dickinson, San Diego, California) equipped with an argon ion laser tuned at 360 nm and detection of emission using a blue fluorescence emission filter (band pass filter of 424/44 nm Ban Pass). FACS analysis results were analyzed using BD CellQuest™ software for Windows XP to determine the ratios of cell cycle phases according to the DNA content of individual cells (2n for phase G1, 4n for G2 and M phases and intermediate values for S phase).

**Determination of the Mitotic index (% of cells in M phase)**

The metaphase/ anaphase index (M/AI) determination in the same sample was carried out by the microscopic examination through counting the proportion of DAPI-stained cells in mitosis (metaphase and anaphase figures) compared to the total number of cells. At least 1000 cells were scored in total in 5 replicates.

4- Fixation and processing for immunofluorescence

Collected samples were fixed in 1mL 4% paraformaldehyde (PFA) for 1 h, RT and washed in PBS (3×10 min). Then the cell wall was digested using 1mL of an enzyme cocktail (2% w/v cellulose, 1% w/v Pectinase, 0.05% w/v Macerozyme, 0.4% w/v Manitol, 1% v/v glycerol and 0.2% v/v Triton X-100, 30 min at 37°C). Finally, samples were washed with 1% v/v glycerol and 0.2% v/v Triton X-100 in PBS (3×10 min). A drop of the cell pellet was placed in a microscope slide covered with poly-lysine and blocked with 2% w/v BSA and 0.05 % v/v Tween in PBS blocking solution, for 30 min at RT. Samples were incubated with the first antibody diluted 1:1000 in blocking solution (Rabbit IgG anti-AtNuc-L1, kindly supplied by Dr. Julio Sáez-Vásquez, CNRS-University of Perpignan, France) for 12 h at 37°C, washed with PBS (3×5 min) and incubated with the second antibody (Alexa Fluor® 488-labeled anti-rabbit polyclonal
antibody, Molecular Probes Cat. No.11001) diluted 1:100, for 3 h at 37ºC, followed by washing with PBS (2×5 min) and counterstained with DAPI (4,6-diamino-2-phenyl-indol), 5µg/µl in PBS, for 5 min. After washing with PBS (2×5 min) and with H2O2 (2×5 min), samples were mounted with DABCO and observed under the Confocal Microscope. Microscopical images were analyzed using the “Leica AF” software to estimate the stained nucleolar area.

5- Statistical analyses

Data were collected from different analyses after each experiment in an Excel datasheet (Microsoft Office 2010). The average, data range and standard deviation in each experiment were estimated. In quantitative studies involving data comparison between different experimental means, data were analyzed according to Steel (1980). Using SPSS v.22 program the variance of differences was statistically analyzed using Student t test. Degree of freedom was followed as p≤0.05 (95%) was considered statistically significant (*).

Results

1- Magnetically Altered Gravity Causes Little Effect on Arabidopsis Cell Proliferation

In order to demonstrate the impact of altered gravity on the cell proliferation rate, the proportion of cells in G1, S and G2/M phases was determined by the means of flow cytometry, i.e. by determination of the DNA content for each individual cell. Results reveal little differences among the altered gravity positions and the external 1g control (Figure 2). Some accumulation of cells in S phase can be appreciated under simulated microgravity (0g* unstable and levitation), with an insignificant reduction in the proportion of cells in G1 phase.

As a complementary approach for the cell proliferation studies, cell division rate was determined by estimating the mitotic index to evaluate the impact of simulated microgravity on Arabidopsis cell proliferation. The mitotic index was estimated by the proportion of cells stained with DAPI relative to metaphase/anaphase mitotic figures. Figure 3 indicates a significant decrease in the mitotic index under simulated microgravity conditions (0g* unstable and levitation) compared
with the external 1g control, while it does not reach significance under the internal 1g* control or Mars (0.37g*) conditions.

2- Arabidopsis Cell Growth is Barely Influenced by exposure to Diamagnetic Levitation

Since the nucleolus is a reliable indicator of the cell growth in proliferating cells (Medina et al 2000), we used a nucleolar protein, AtNucL1, to quantify the nucleolar area, in order to detect the effect of altered gravity levels generated by diamagnetic levitation on the Arabidopsis cell growth and nucleolar activity (Figure 4). Statistical analyses reveal a general reduction in all magnetic field samples versus the external 1g control, but the nucleolus area reduction reaches statistical significance only under the unstable altered microgravity conditions (0g*/unstable), compared with the external 1g control.

3. Use of 0g* levitation conditions to expose cells to simulated microgravity: Do cells actually levitate inside a levitated droplet of cell suspension?

The use of magnetic levitation as a means of exposing living beings to simulated microgravity conditions is based on the consideration that living beings levitate under a diamagnetic force whose magnitude is close to the levitation point of water. Suspension cell cultures are an excellent model system to test whether or not the response of living matter to the magnetic force in terms of levitation is closely enough to that of water, since our system it is composed of cells suspended in a medium basically consisting of water.

For this purpose, we have designed an experiment consisting of the levitation of a droplet of cell suspension inside the magnet bore using the magnetic levitation point of water (Figure 5). The visual observation of the behavior of cells inside the droplet during levitation will allow us to determine whether the cells are experiencing the same or a differential magnetic force as the surrounding media, leading in the latter case to sedimentation of the cells.

In the experiment, an Arabidopsis cell suspension droplet was installed inside the magnet bore to stabilize the levitation (0g* stable levitation position) in the presence of a video camera (See images of video captures in Figure 5 and the whole video clip as Supplementary material 1). When the images were taken from the top of the magnetic bore, the cells seemed to be uniformly distributed within the drop. However, we introduced a side mirror to get at the same time a top
and a lateral view of the levitating droplet (Figure 5A), Using this setup, we observed that the Arabidopsis cell suspension droplet was introduced into the levitation region, appearing floating in air under stable levitation (Figure 5B1, B2). Increasing the magnetic force up to 16T, produced the exclusion of the cell suspension droplet out of the magnet bore or its projection against the bore wall after escaping out of the levitation zone (Figure 5B3). A detailed observation of the droplet under stable levitation conditions using the lateral mirror showed that cells were not equally distributed throughout the volume of the drop, neither they were placed at the center of the drop, but they appeared sedimented at the bottom of the levitated drop (Figure 5B4). The cell movements inside the levitated drop clearly showed that the cells were experiencing a non-strong enough magnetic levitation force to keep them in suspension. From time to time, a few individuals cells were observed to levitate (Figure 5B5), but most of the cells remained at the bottom of the droplet.

Discussion

Exposing suspension cell cultures to simulated microgravity conditions (diamagnetic levitation) produces alterations in the cell cycle and ribosome biogenesis (as determined by the nucleolus size estimation after anti-nucleolin detection), which are compatible with the effects on cell proliferation and cell growth previously observed in meristematic cells of seedlings when they were exposed to real or simulated microgravity (Manzano et al 2013, Matía et al 2010). While ribosome biogenesis (a marker of cell growth) was depleted by unstable simulated microgravity (0g*/unstable), the increase in the proportion of cells at the S phase of the cell cycle, together with the reduction in the mitotic index under both simulated microgravity settings (0g*/unstable and 0g*/levitation), suggested an increase in cell proliferation. On the other hand, the observation of smaller nucleoli under unstable microgravity conditions is consistent with the reduction in the proportion of cells in G2 phase, known to have large and active nucleoli (González-Camacho & Medina 2006). Although these results are in agreement with those found in real microgravity (Matía et al 2010) and the differences between internal 1g* and external 1g controls were not significant, it must be noticed that most of the observed variations were also not significant in simulated microgravity versus internal 1g* control maybe due to the short duration of the treatment in relation to the duration of the cell cycle.
Therefore, the essential question is to correlate these alterations to the change in the net gravity force or to the presence of a high magnetic field, or to a combination of the two factors. In our experiments we used the diamagnetic force required to compensate the weight of the cell suspension as a whole (13T with a strong gradient), but it was noticed that the cells inside the droplet sedimented due to the different densities and magnetic susceptibilities of components other than water. In the levitating droplet experiment sedimentation occurs because the gravity force is higher than the magnetic force applied to the cells. The reason is that the magnetic susceptibility is lower for the cells than for water, so that the magnetic field acting on the cells \( F_{m_{\text{cells}}} \) is lower than the one acting on the whole solution \( F_{m_{\text{sol}}} \), which is equal to the gravity force on the levitation point. Moreover, it is noticed that the cells were not expelled out of the water drop, but they were kept inside it. According to the formula for diamagnetic levitation, three scenarios can be described to levitate a cell suspension, as depicted in the scheme shown in Figure 6:

- **0g* stable levitation (calculated for solution):** It is the configuration we used for our levitating droplet experiment. \( F_{m_{\text{sol}}} \) is equal to \( g \) in the center of the droplet that it is stabilized by a slightly lower \( F_{m_{\text{sol}}} \) in the top and slightly higher \( F_{m_{\text{sol}}} \) in the bottom of the drop. Empirical value of \( F_{m_{\text{sol}}} \) is quite similar to the calculated value of \( F_{m_{\text{H}_2\text{O}}} \) for pure water. \( F_{m_{\text{sol}}} > F_{m_{\text{cells}}} \), so sedimentation occurs.

- **0g* unstable levitation (calculated for solution):** It is performed in the secondary 0g* point in a non-levitation condition configuration. \( F_{m_{\text{sol}}} \) is still equal to \( g \) in the center of the sample but it is non-stable due to slightly higher, repelling \( F_{m_{\text{sol}}} \) in both the top and the bottom of the cell culture, facilitating the suspension to be expelled. \( F_{m_{\text{sol}}} > F_{m_{\text{cells}}} \), so sedimentation occurs.

- **0g* “stable” levitation (calculated for the cells):** It is a “virtual” experiment we have not attempted due to the complexity of both its performance and interpretation. If we are able to tap the culture container preventing the liquid to escape, an increase in the magnetic field could be such that \( F_{m_{\text{cells}}} \) is equal to \( g \) in the center of the sample. \( F_{m_{\text{sol}}} > F_{m_{\text{cells}}} \) so movements in the fluid will produce shear stress by fluid motions, but the cells will be “stabilized” in the center of the culture by slightly lower \( F_{m_{\text{cell}}} \) in the top and slightly higher \( F_{m_{\text{cell}}} \) in the bottom of the container. Consequently, if the B dB/dz conditions can be established, considering the magnetic properties of the cells only,
then the cells could be “theoretically” levitated inside a non-levitating solution. In fact, during the preliminary tests to set up our experiments conditions we detected that increasing the magnet force to 16T was enough to eliminate the water droplet out of the levitation range without a cap.

Therefore, we arrive to the conclusion that using the magnetic levitation in suspension cultures is not recommended for microgravity simulation due to technical constrains. In fact, previous experiments with 'levitated' E coli cell cultures revealed some pitfalls of doing liquid culture experiments in gradient magnetic field including a reduced, but still present, sedimentation rate (Dijkstra et al 2011). Precisely, in the conditions of the levitating droplet experiment, the cells were exposed to rather low-gravity levels by the magnetic force (our estimation is a residual g force <0.05g), but not enough to maintain the cells in suspension. Consequently we have to conclude that magnetic levitation is not a system of choice for microgravity simulation. In addition to the residual gravity level which has been evidenced in our experiment, we have to take into account the considerable secondary effects of the high magnetic fields, which become evident in the 1g* internal control. These secondary effects make difficult to reach unequivocal relationships between the observed results and the effects of microgravity environment, as it occurs in a spaceflights or free-fall experiments. In combination with previous results from our group, in which we found problems in defining 1g control conditions to expose plant cell suspension cultures to simulated microgravity in 2D-pipette-clinostats (Kamal et al 2015), we consider that the immobilization of cell cultures to be used in mechanical facilities, such as conventional clinostats or the RPM appears as the most suitable and reliable alternative for long-term microgravity simulation experiments in this biological system.

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Supplementary Material 1. Videoclip demonstrating that the cells cannot levitate at the same point than the droplet. A lateral mirror provides us a lateral view of the droplet that normally is observed from the top of the magnetic bore.

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FIGURE CAPTIONS

Figure 1: Magnetic levitation set up. A) Photo of the water-cooled duplex-Bitter magnet located at HFML with the samples positioned inside the magnet bore. The temperature is controlled by a double-walled metal tube connected to a 22°C water bath. A PVC spacer is used to place the stack of samples in the correct position. B) The samples are contained in 26.25 mm high tubes placed on top of each other at four effective *g* levels. The space between the samples was 26.25 mm and all samples were in the dark before and during the experiment (no light reached the magnet bore). C) Closer view of a sample tube, 1 ml of MM2d suspension cultures into the tube (layer 1-2mm) to ensure a similar force throughout the whole biological samples. D) Profile of the magnetic flux density (*B*) and the magnetic levitation force along the magnet bore. The samples were placed symmetrically in relation to the centre of the bore (195 mm above the top) indicated in the graph by vertical lines (straight lines for 0g* levitation, 0g* unstable, 0.37g* and 1g*). The red curve shows the magnetic flux density as a function of the vertical position (z) in the magnet. The blue curve indicates the product of the field strength *B*(z) and the field gradient (*B´*(z) = dB/dz), which is the derivative of the field strength with respect to the vertical position. The corresponding value of the effective gravity is equal to *g* (1 + *B*(z) *B´*(z)/1360), so a magnetic force of -1360 T²/m is able to levitate water. The magnetic flux density is shown for the four experimental g levels and also for the external 1g control (at some meters distance from the magnet).

Figure 2: Arabidopsis cell cycle phase distribution after magnetic levitation experiment for 3 hours. A) Flow cytometry analysis in which each panel represents the relative number of cells according to the DNA content in each cell for any g level as explained in Materials and methods. First peak (2n) reflects G1 phase and the second peak (4n) reflects G2/M phase. B) DNA content histogram of the same samples in which the peaks have been quantified for the different cell cycle phases.

Figure 3: Cell division figures induced by magnetic levitation for 3 hours experiment. Metaphase /Anaphase cells (M/A) index was determined as the proportion mitotic cell per the rest of population. Significant differences versus the external 1g control are shown, P-Value < 0.05 (*). (*) in g levels refers to the magnetic field induced this simulated gravity. A baseline effect of the magnetic field at the 1g* position is indicated with a horizontal line.

Figure 4: Nucleolar area under magnetic levitation 3 hours experiment. More than 50 nucleolus areas (α-nucleolin staining) of Arabidopsis cells were measured for each experimental condition. Significant differences versus the external 1g control are shown as means ±S.E.M., P-Value < 0.05 (*). (*) in g levels refers to the magnetic
field induced this simulated gravity. A baseline effect of the magnetic field at the 1g* position is indicated with a horizontal line.

**Figure 5: Arabidopsis cell suspension droplet levitation.** Cell droplet is levitated using diamagnetic levitation instrument. A) Experimental design for the droplet levitation video record using a side mirror for the 2D video record. B) Cell droplet images extracted from the video (Supplementary material 1) show different statement of the droplet levitation and the cells behavior during the levitation.

**Figure 6: Forces acting on three magnetic levitation experimental scenarios.** A) 0g* stable levitation position for cells (theoretical), B) 0g* stable levitation position for suspension (droplet). C) 0g* unstable levitation position (for suspension). Both cells and solvent inside the droplet are exposed to two forces, the variable magnetic force (Fm) and the constant gravity force (g). Corresponding to the density and the particles magnetic susceptibility (\( \chi \)) in the formula, it is reflected that the net force affecting the water or cells droplets is zero due to the force compensation (Magnet and Gravity), whereas it is a residual gravity force for the cells with lower magnetic susceptibility (\( \chi \)) than water, leading to sedimentation within the droplet. Theoretically, it is possible to perform a levitation experiment with cells in which water cannot escape with a cap. Fm\textsuperscript{sol} refers to suspension culture (water + cells) and Fm\textsuperscript{cells} to cells only. Note than in the unstable condition, the solution should form an inverted meniscus due to “escaping” force of water although we could not record that position at the levitation magnet experiment. Neither of three 0g* conditions is equal to the real microgravity ones, stressing the requirement of Space Biology experiments to be confirmed on Spaceflight conditions.
**Figure 1: Magnetic levitation set up.** A) Photo of the water-cooled duplex-Bitter magnet located at HFML with the samples positioned inside the magnet bore. The temperature is controlled by a double-walled metal tube connected to a 22°C water bath. A PVC spacer is used to place the stack of samples in the correct position. B) The samples are contained in 26.25 mm high tubes placed on top of each other at four effective \( g^* \) levels. The space between the samples was 26.25 mm and all samples were in the dark before and during the experiment (no light reached the magnet bore). C) Closer view of a sample tube, 1ml of MM2d suspension cultures into the tube (layer 1-2mm) to ensure a similar force throughout the whole biological samples. D) Profile of the magnetic flux density (B) and the magnetic levitation force along the magnet bore. The samples were placed symmetrically in relation to the centre of the bore (195 mm above the top) indicated in the graph by vertical lines (straight lines for \( 0 \) \( g^* \) levitation, \( 0 \) \( g^* \) unstable, \( 0.37 \) \( g^* \) and \( 1 \) \( g^* \)). The red curve shows the magnetic flux density as a function of the vertical position (z) in the magnet. The blue curve indicates the product of the field strength \( B(z) \) and the field gradient (\( B'(z) = dB/dz \)), which is the derivative of the field strength with respect to the vertical position. The corresponding value of the effective gravity is equal to \( g (1 + B(z) B'(z)/1360) \), so a magnetic force of \(-1360 \) T²/m is able to levitate water. The magnetic flux density is shown for the four experimental \( g \) levels and also for the external \( 1 \) \( g \) control (at some meters distance from the magnet).
Figure 2: *Arabidopsis* cell cycle phase distribution after magnetic levitation experiment for 3 hours. A) Flow cytometry analysis in which each panel represents the relative number of cells according to the DNA content in each cell for any g level as explained in Materials and methods. First peak (2n) reflects G1 phase and the second peak (4n) reflects G2/M phase. B) DNA content histogram of the same samples in which the peaks have been quantified for the different cell cycle phases.
Figure 3: Cell division figures induced by magnetic levitation for 3 hours experiment. Metaphase /Anaphase cells (M/A) index was determined as the proportion mitotic cell per the rest of population. Significant differences versus the external 1g control are shown, P-Value < 0.05 (*). (*) in g levels refers to the magnetic field induced this simulated gravity. A baseline effect of the magnetic field at the 1g* position is indicated with a horizontal line.
Figure 4: Nucleolar area under magnetic levitation 3 hours experiment. More than 50 nucleolus areas (α-nucleolin staining) of Arabidopsis cells were measured for each experimental condition. Significant differences versus the external 1g control are shown, P-Value < 0.05 (*). (*) in g levels refers to the magnetic field induced this simulated gravity. A baseline effect of the magnetic field at the 1g* position is indicated with a horizontal line.
Figure 5: *Arabidopsis* cell suspension droplet levitation. Cell droplet is levitated using diamagnetic levitation instrument. A) Experimental design for the droplet levitation video record using a side mirror for the 2D video record. B) Cell droplet images extracted from the supplementray material video 1 shows different statement of the droplet levitation and the cells behavior during the levitation.
Figure 6: Forces acting on three magnetic levitation experimental scenarios. A) $0g^*$ stable levitation position for cells (theoretical), B) $0g^*$ stable levitation position for suspension (droplet). C) $0g^*$ unstable levitation position (for suspension). Both cells and solvent inside the droplet are exposed to two forces, the variable magnetic force ($F_m$) and the constant gravity force ($g$). Corresponding to the density and the particles magnetic susceptibility ($X$) in the formula, it is reflected that the net force affecting the water or cells droplets is zero due to the force compensation (Magnet and Gravity), whereas it is a residual gravity force for the cells with lower magnetic susceptibility ($X$) than water, leading to sedimentation within the droplet. Theoretically, it is possible to perform a levitation experiment with cells in which water cannot escape with a cap. $F_{m_{sol}}$ refers to suspension culture (water + cells) and $F_{m_{cells}}$ to cells only. Note than in the unstable condition, the solution should form an inverted meniscus due to “escaping” force of water although we could not record that position at the levitation magnet experiment. Neither of three $0g^*$ conditions is equal to the real microgravity ones, stressing the requirement of Space Biology experiments to be confirmed on Spaceflight conditions.