Arabidopsis thaliana root growth under simulated microgravity

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Introduction

In the context of the Space conquest to Mars, survival of astronauts will be ensure by **Life Support Systems** which provide water, oxygen and nutrition to the crew. In these systems, plants will play an essential role. Therefore, their adaptation to this new space environment will determine the success of this challenge.

Among all the factors related to space environment, we focus on **gravity**. Since they emergence in Paleozoic era, land plants have only developed under 1*g* constant gravity. Thus, it is essential to understand both, **how plants will respond to altered gravity** and **what will be the consequences of this adaptation on their growth**.

Objective

We have studied **the impact of simulated microgravity (µg) on root growth**, mainly by cell division and elongation, in the model plant *Arabidopsis thaliana*.

Materials and methods

Plant material: *Arabidopsis thaliana ecotype Col-0.* Wild Type and transformed with *CYCB1::GUS*[1].

Microgravity simulator: Horizontal 2D clinostat[2]. The angular velocity was 2 rpm and the level of simulated microgravity was 10^{-4}*g*.

Culture *conditions*: *Seeds surface* were sterilized and they were sown on *Murashige and Sko*og medium with 1% sucrose in µg or 1*g* environment. Seedlings were cultivated during 6 days under a photoperiod of 18 h of light at 22°C and 6 h in dark at 18°C.

Methods of analysis: DIC microscopy, RT-qPCR[3], flow citometry[4].

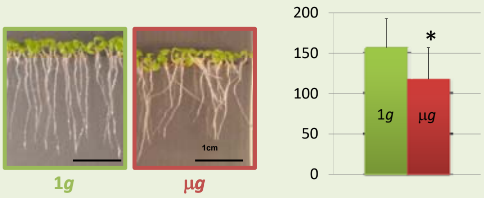
Computer resources: ImageJ (NeuronJ), SPSS 22.

Clinostat 2D

Results

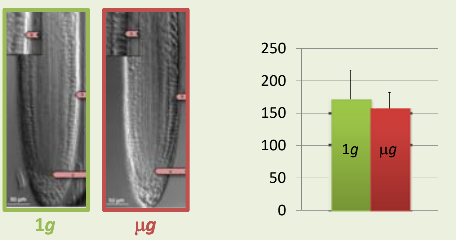
Morphometric analysis of roots

• **Root length (mm)**



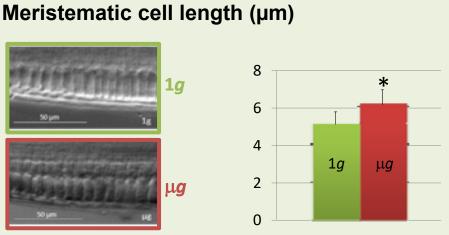
Microgravity inhibits root growth and changes root orientation.

• **Meristem length (µm)**



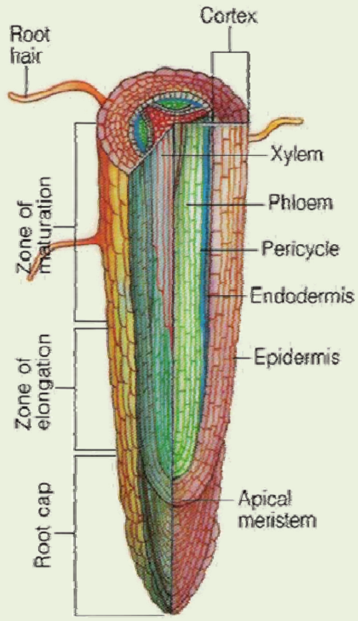
Microgravity does not affect significantly meristem length.

• **Meristematic cell length (µm)**

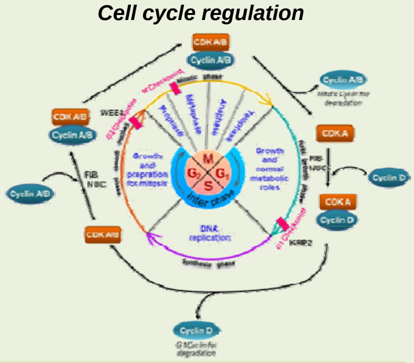


Microgravity increases the length of meristematic cells.

Arabidopsis thaliana root structure

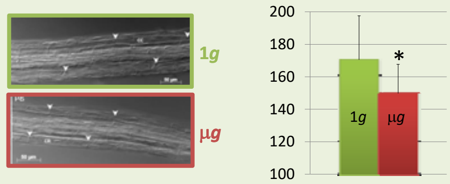


Cell cycle regulation

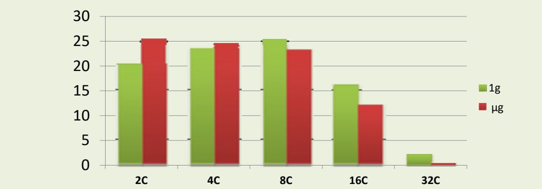


Zone of maturation

• **Differentiated cells length (µm)**



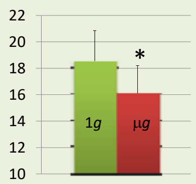
• **Rate of ploidy of differentiated cells (%)**



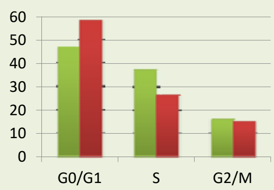
Microgravity inhibits cell size (elongation) and reduces ploidy rate.

Apical meristem

• **Proliferation rate (cell / 100 µm)**

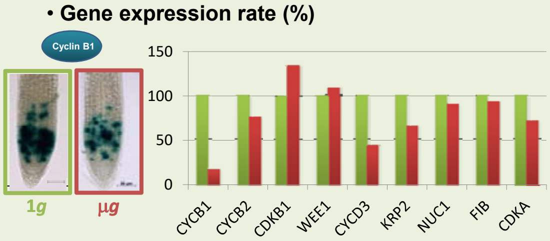


• **Cell cycle phases (%)**



In microgravity, meristematic cells decrease their division and the most of them stay in G1 phase instead of moving to the S phase.

• **Gene expression rate (%)**



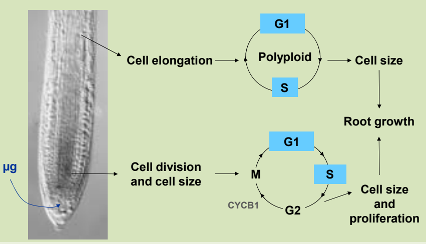
Microgravity decreases cell proliferation (CDKA) acting on the transition G1 / S (CYCD3 and KRP2) and G2 / M (CYCB1, CYCB2, CDKB1 and WEE1) of the cell cycle.

Conclusions and prospects

Gravity affects many biological process related to plant growth and development:

✓ In simulated microgravity: root length is smaller and root apex cells are longer but their proliferation is lower, keeping stable the size of the meristem.

✓ Microgravity modifies cell cycle via gene expression, arresting the cycle in G0 / G1. This reduction elicits a decrease of the ploidy in differentiated cells and their length. Therefore, all these alterations may explain the reduction of root size.



❑ A broad genome study with DNA arrays should be carry out.

❑ Is the mechanism of oxidative stress triggered by microgravity similar to those involved in other abiotic stresses?

❑ These experimental results should be compared with the real microgravity in spatial experiment.

Acknowledgments

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References

1. Colon-Carmona *et al.* (1999) Spatio-temporal analysis of mitotic activity with a labile cyclin-GUS fusion protein. *Plant Journal*, 20:503-8
2. Aarrouf *et al.* (1999) Changes in hormonal balance and meristematic activity in primary root tips on the slowly rotating clinostat and their effect on the development of the rapeseed root system. *Physiol Plant*. 105:708-18
3. Based on the protocol of Super Script II ™, Invitrogen
4. Based on the protocol of Ulrich & Ulrich (1991). High-resolution flow cytometry of nuclear DNA in higher plants. *Protoplasma*, *165:*212-215.