MELISSA
MICRO-ECOLOGICAL
LIFE SUPPORT SYSTEM
ALTERNATIVE

CREATING
A CIRCULAR
FUTURE

SEEDLING GROWTH

results from the largest ESA/NASA Arabidopsis experiment on the ISS looking into the molecular ADAPTATION OF PLANTS TO THE MOON GRAVITY and other life support system relevant scenarios

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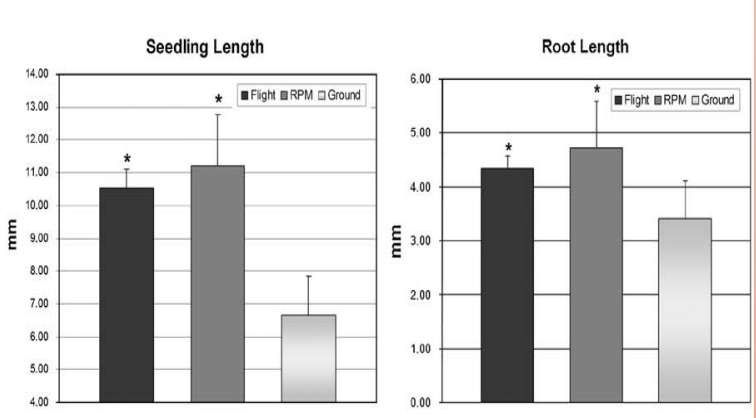
NASA

MICROGRAVITY AFFECTS CELL GROWTH AND CELL PROLIFERATION IN PLANT ROOT CELLS

- The **"ROOT"** experiment was performed in the ISS in the course of the Spanish "Cervantes" Soyuz Mission and a ground control experiment was done in a **Random Positioning Machine** (RPM).
- The experiments used seedlings of *Arabidopsis thaliana* ecotype Columbia, which grew at 22^{0}C **in darkness**, for 4 days.
- The experiment aimed to know the effects of real and simulated microgravity on nuclear structure and function in root meristematic cells.

Seedling and Root Length



In the experimental conditions, seedlings and roots were significantly longer than in the 1g ground controls.

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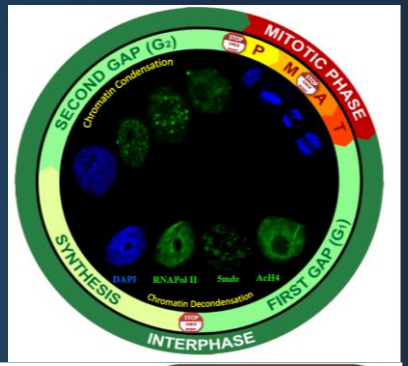
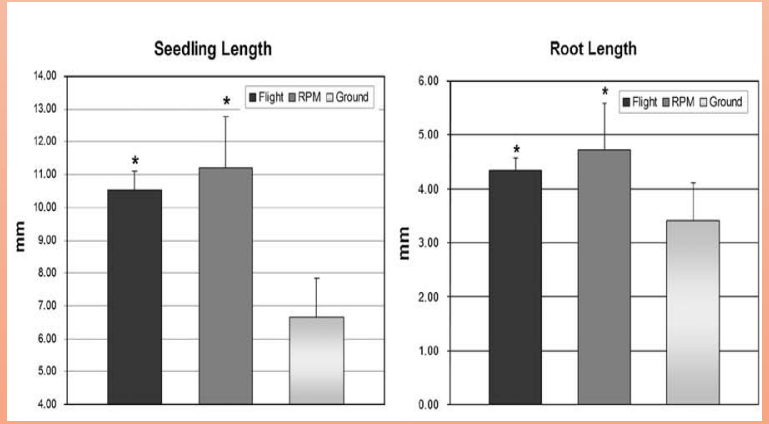


Seedling and Root Length



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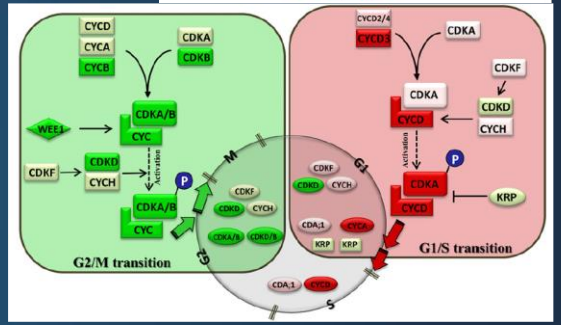
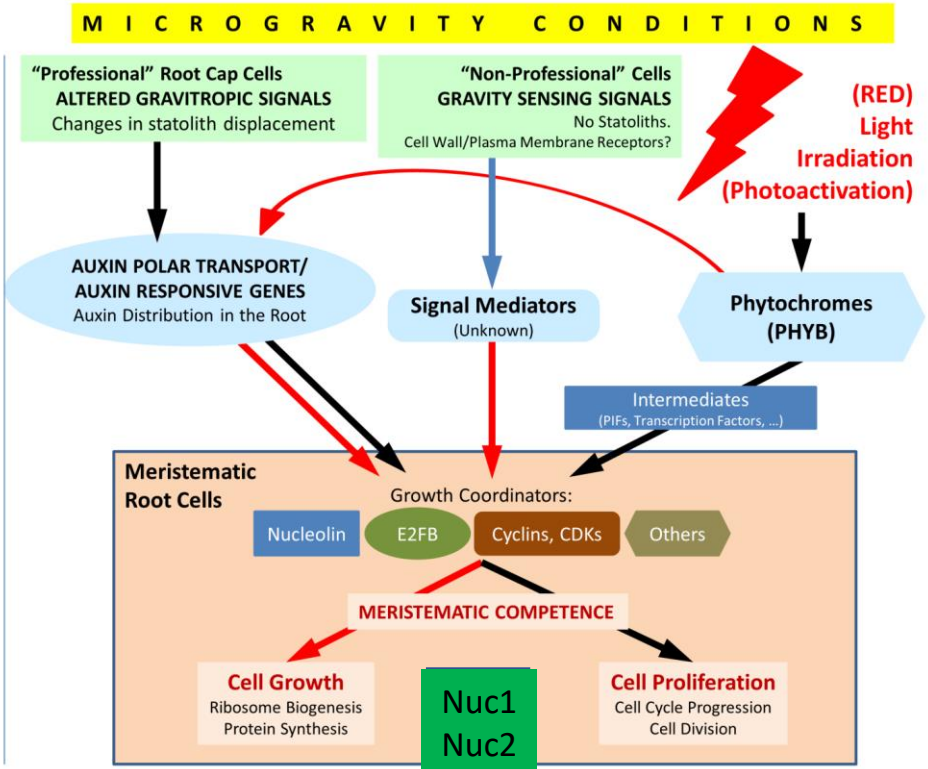
Cell Cycle control seems to be affected by simulated microgravity



Life Support Systems will benefit of the basic understanding of this phenomena

The Seedling Growth research experiments on ISS
Light and gravity interaction

Two genotypes and Three collection of mutants
to dissect how the cells integrate the signals



Aux1.7
Eir1.1
Tir1

Auxin signalling Collection

(defective gravitropism)

PhyA
PhyB
Ler

Phytocrome Collection

(defective phototropism)

Nuc1
Nuc2
Col0

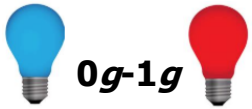
Cell Growth collection
(Defective growth / stress response)

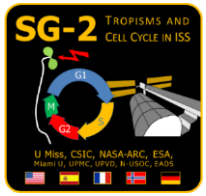
The Seedling Growth research experiments on ISS
Light and gravity interaction

Seedling Growth-1 (SG1)

Launch SpX-2	01 March 2013
Experiment ISS	22 March – 24 May 2013
Recovery SpX-3	18 May 2014

- Analysis of phototropism in microgravity conditions.
- Influence of phototropic alteration on cell growth and proliferation.



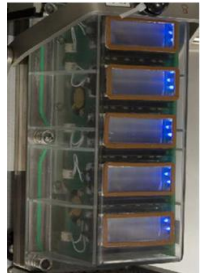


Seedling Growth-2 (SG2)

Launch SpX-4	21 Sept 2014
Experiment ISS	01 Nov – 15 Dec 2014
Recovery SpX-5	11 Feb 2015

- Analysis of phototropism in conditions of fractional gravity (Moon, Mars).
- Effects of red light photoactivation on cell cycle and ribosome biogenesis and their regulation by auxin. Studies of gene expression.

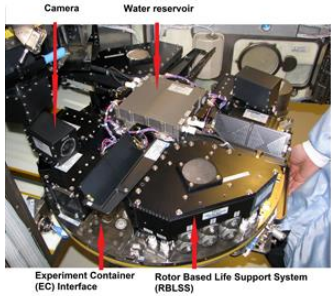
TROPI cassettes

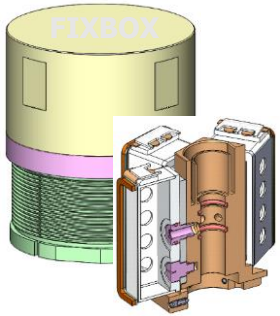


Seedling Growth-3 (SG3)

Launch SpX-11	3 June 2017
Experiment ISS	13 June – 25 June 2017
Recovery SpX-11	4 July 2017

- Effects of red light photoactivation on cell cycle and ribosome biogenesis and their regulation by auxin. Studies of cell biology by microscopic observation.
- Studies of gene expression and cell biology in conditions of fractional gravity (Mars).



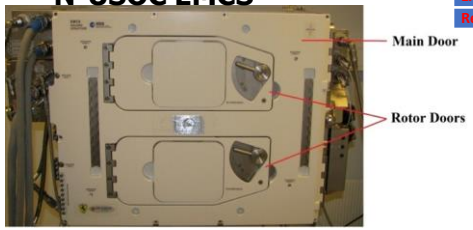


Seedling Growth-3 (Ground Reference test – September 2018)

Preparation AMES-RC	August 2018
Experiment N-USOC	4 September – 17 September 2018
Recovery Madrid	20 September 2018

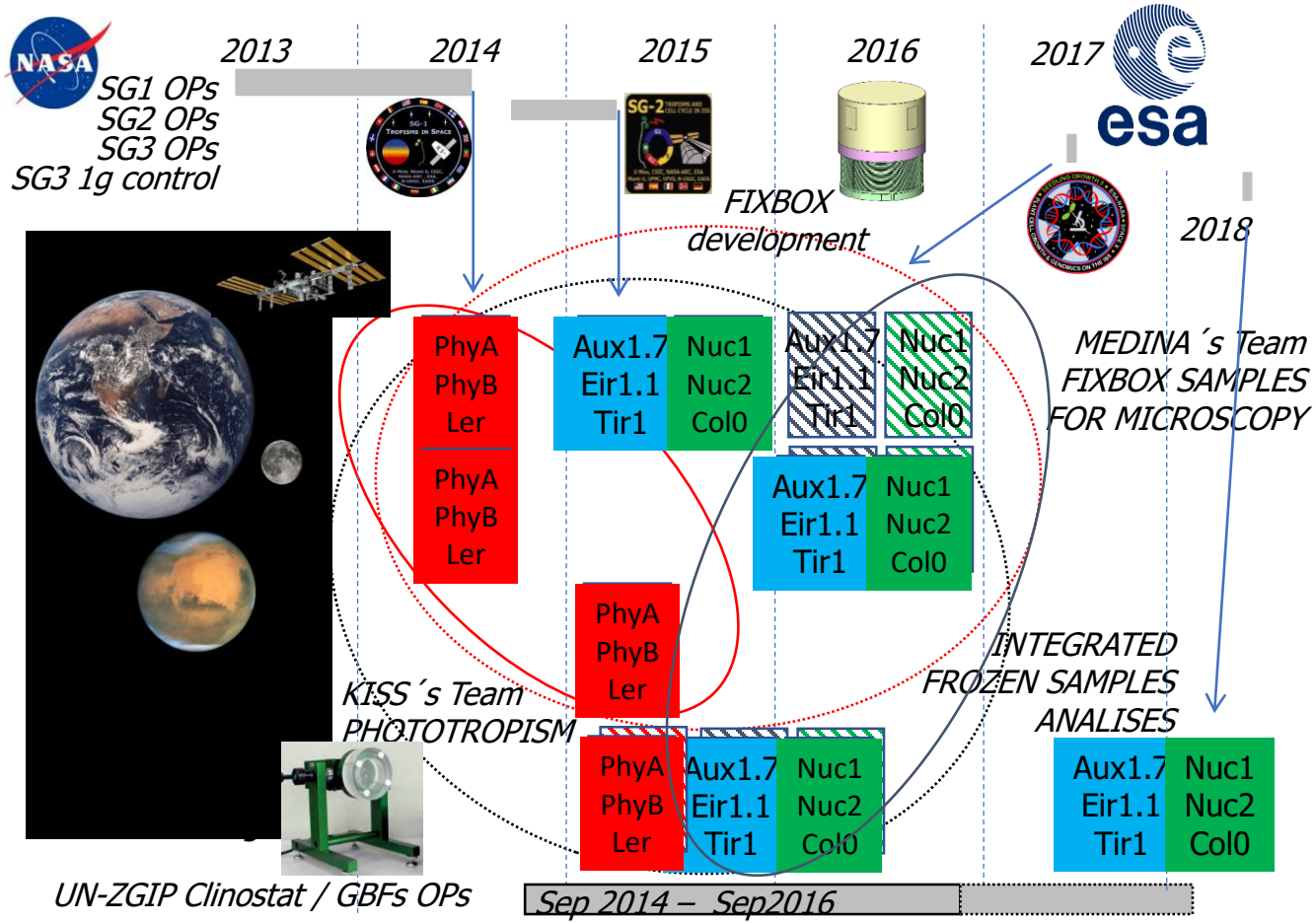
- Ground reference test to check ISS conditions different from g level.

N-USOC EMCS

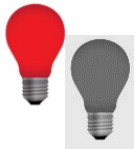


Manzano et al. Microgravity Science and Technology. 32, 1105–1120 (2020).

Distribution of mutant collections by *g* level in the Seedling Growth Series



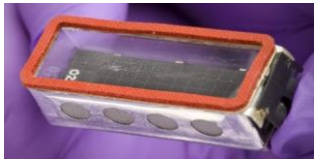
Collection RNA-seq results from the Seedling Growth Series

PhyA PhyB Ler		***20 Samples (6 conditions x 3-4 replicates)***	***BLUE LEDs***	***x***	***µg (ISS) <0.1g Moon g Mars g .57g***	***x***	***Wt (Ler)***	***GLDS 251***
PhyA PhyB Ler		***36 Samples (6 conditions x 3-4 replicates)***	***BLUE LEDs***	***x***	***µg (ISS) <0.1g Moon g Mars g .57g 1g (ISS)***	***x***	***PhyA PhyB***	***GLDS 346***
Nuc1 Nuc2 **Col0**		***62 Samples (8 conditions x 3 mutants x 2-3 replicates)***	***DARKNESS RED LEDs***	***x***	***1g (ISS) 1g (GRT) µg (ISS) 0.3g (ISS)***	***x***	***Wt (Col0) Nuc-1 Nuc-2***	***GLDS 313 GLDS 314***
Aux1.7 Eir1.1 Tir1 Col0		***64 Samples (8 conditions x 4 lines x 2 replicates)***	***DARKNESS RED LEDs***	***x***	***µg (ISS) 0.3g (ISS) 1g (ISS) 1g (GRT)***	***x***	***Aux1.7 Eir1.1 Tir1 Col0***	

Space Omics
An esa funded Topical Team

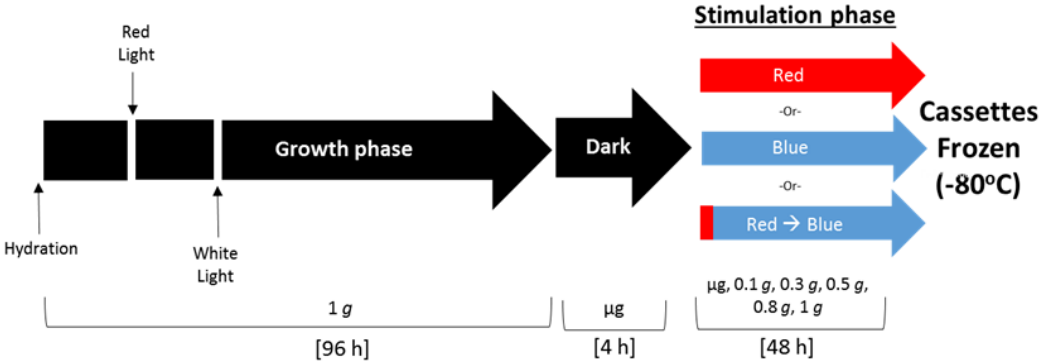
International Standards for Space Omics Processing ISSOP
GeneLab
Open Science for Exploration

The Seedling Growth research experiments on ISS
Light and gravity interaction

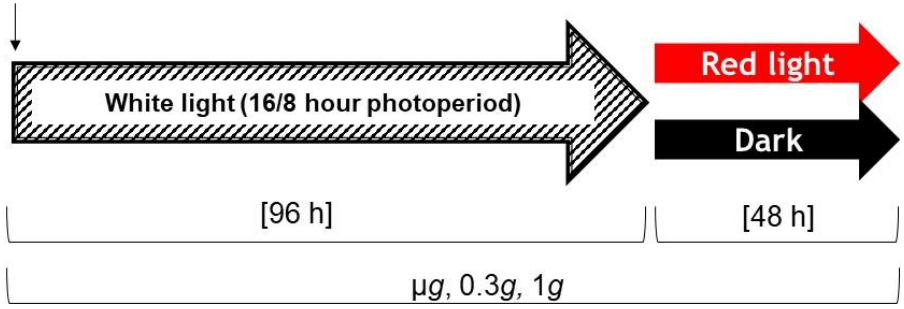


Light stimulation during the Growth Procedure:

Arabidopsis thaliana ecotype
"Landsberg erecta"
Genotypes:
Wild Type (Ler)
Mutant for phytochrome A *(phyA)*
Mutant for phytochrome B *(phyB)*



Arabidopsis thaliana ecotype
"Columbia"
Genotypes:
Wild Type (Col0)
Three mutants for auxin transport:
(eir1.1, tir1, aux1.7)
Two mutants for the nucleolar protein nucleolin:
(nuc1, nuc2)

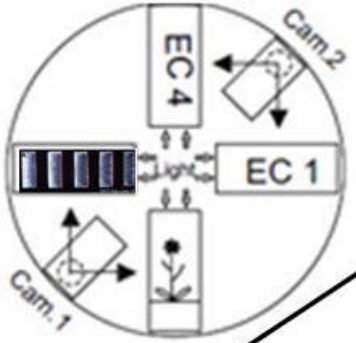


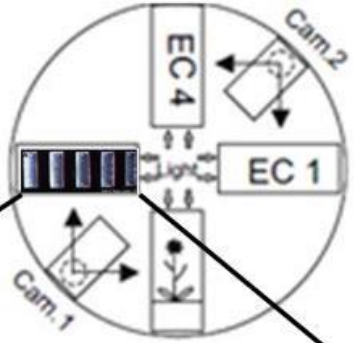
Sample preservation was DEEP FREEZING (-80°C) for transcriptomics

The Seedling Growth research experiments on ISS
Light and gravity interaction

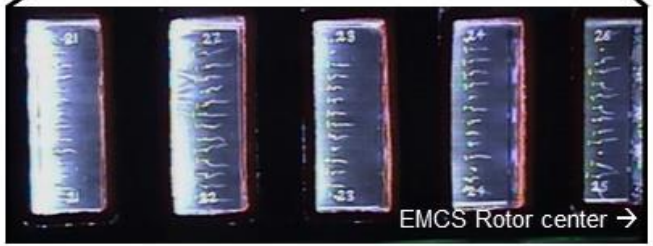
Different centrifuge speeds on board EMCS to provide a 0*g* to 1*g* gradient

Clustering of the samples to minimize g level differences from the Experimental container geometry when pooling samples was considered





Microg



Low g

Reduced Earth g level

Moon g

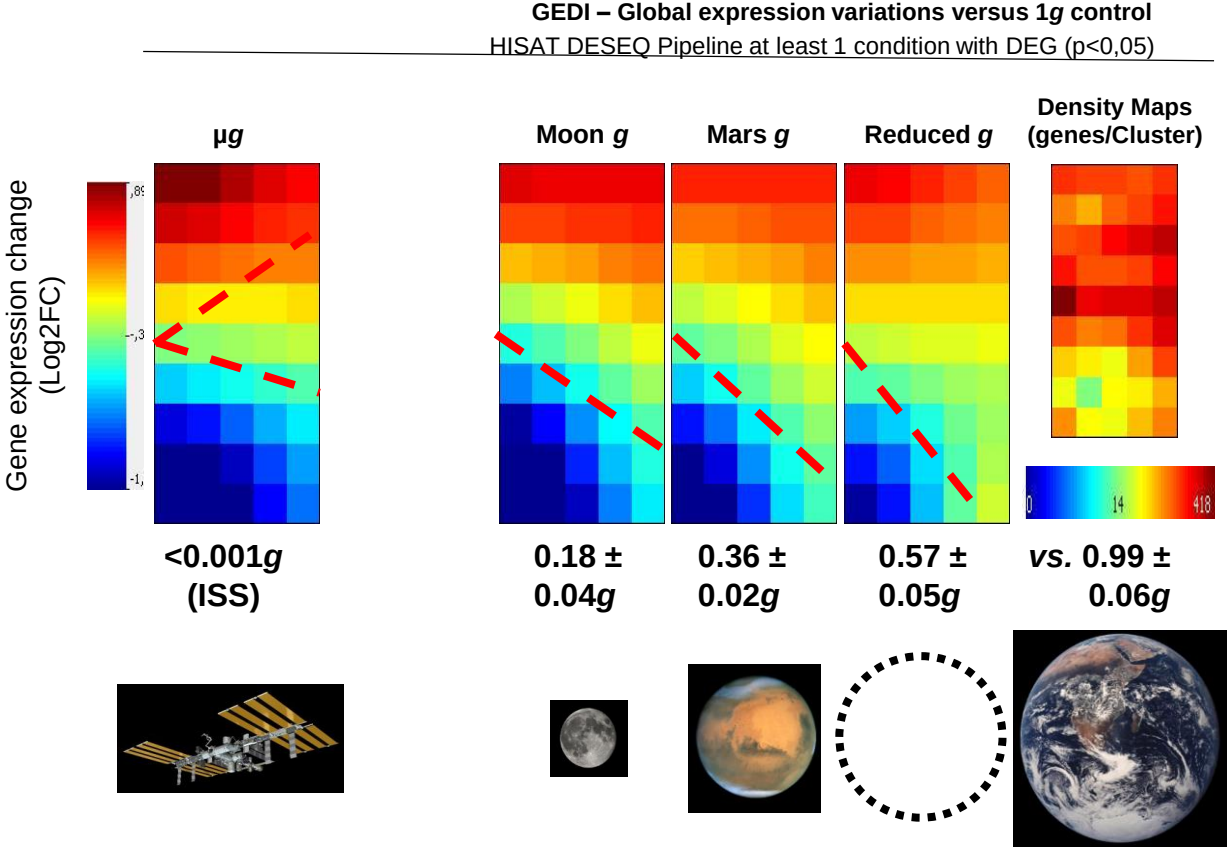
1g control

Distance from seedling cassette center to the EMCS center (mm)	270	238	206	174	142
Nominal 0.1*g*	**0.13**	**0.11**	0.10	0.08	**0.07**
Nominal 0.3*g*	**0.39**	**0.34**	0.30	0.25	**0.21**
Nominal 0.5*g*	**0.65**	**0.58**	0.50	0.42	**0.34**
Nominal 0.8*g*	**1.05**	**0.92**	0.80	0.68	**0.55**

Mars g

PhyA
PhyB
Ler

Global expression profile (RNA-seq) effects are similar in reduced gravity samples, decreasing with gravitational load…



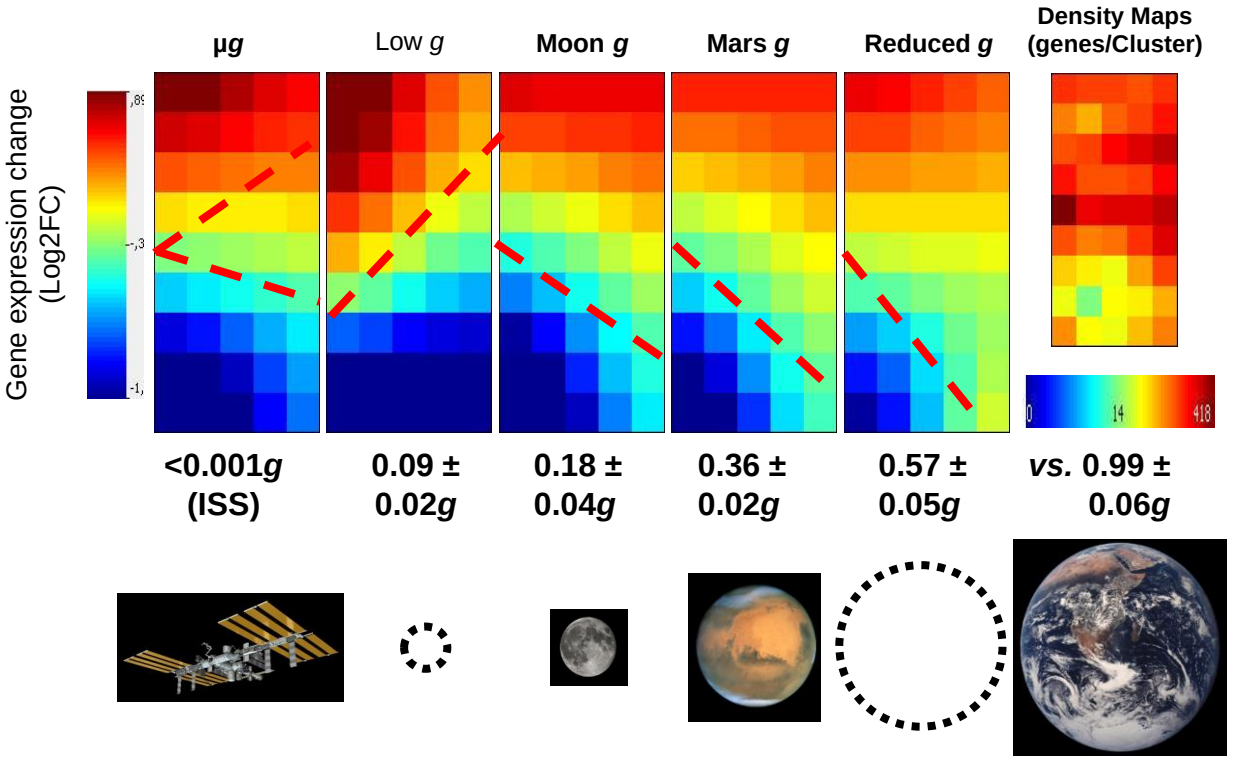
Herranz et al. Frontiers in Plant Science 2019. DOI 10.3389/fpls.2019.01529

PhyA
PhyB
Ler

Global expression profile (RNA-seq) effects are similar in reduced gravity samples, decreasing with gravitational load except in Low-g conditions

GEDI – Global expression variations versus 1*g* control

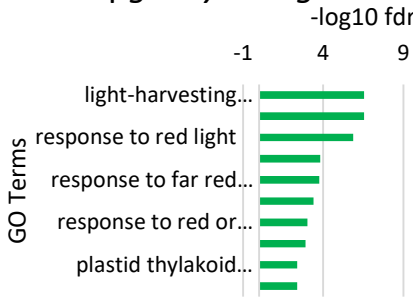
HISAT DESEQ Pipeline at least 1 condition with DEG (p<0,05)

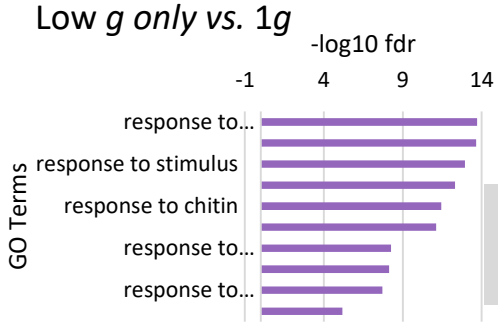


Herranz et al. Frontiers in Plant Science 2019. DOI 10.3389/fpls.2019.01529

PhyA
PhyB
Ler

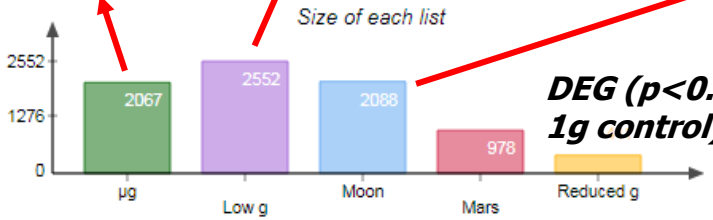
Complex response to partial g on the ISS in blue photostimulated plants



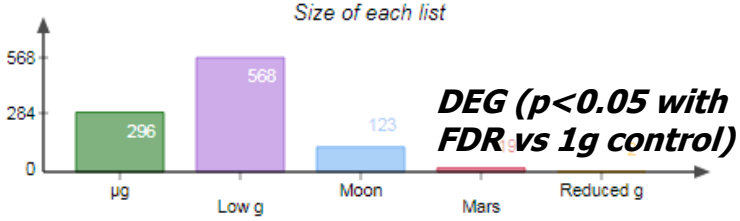


Powerful and general stress response- Conflict between tropisms?

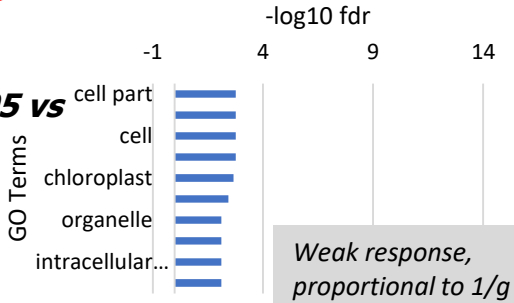
Altered phototropism pathways - Compensating gravitopism?



DEG (p<0.05 vs 1g control)



DEG (p<0.05 with FDR vs 1g control)



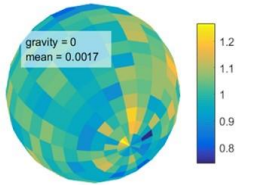
Weak response, proportional to 1/g (cell structure) -gravirresistance by organelle retrograde signalling?

Herranz et al. Frontiers in Plant Science 2019. DOI 10.3389/fpls.2019.01529

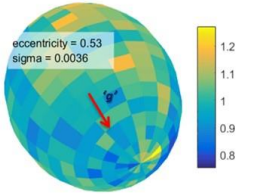
PhyA
PhyB
Ler

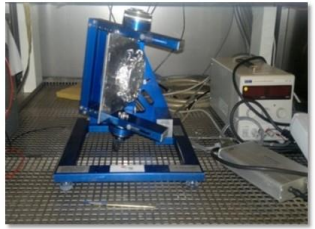
Similar effects at low gravity levels (moon) has been also observed at the cell nucleolus level (stress sensor) in simulated fractional gravity experiments

A) Simulated μg RPM paradigm

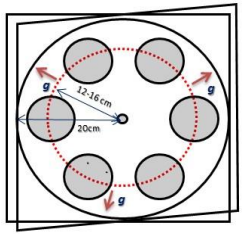


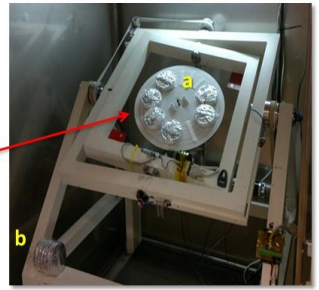
B) Simulated partial *g* RPM^{SW}

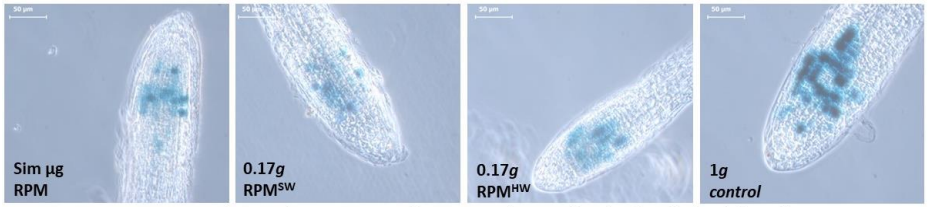




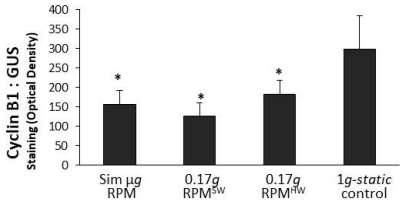
C) Simulated partial *g* RPM^{HW}

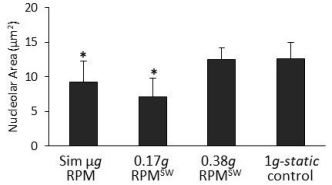


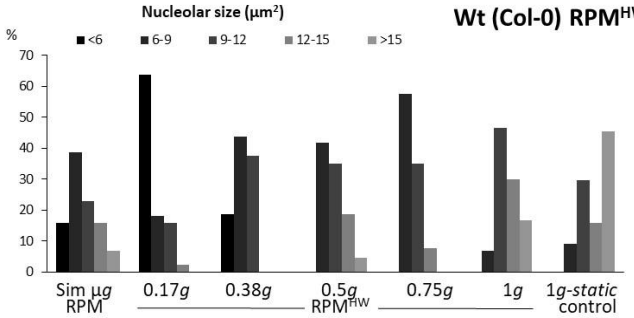




Root meristems from Cyclin B1 : GUS seedlings exposed to simulated reduced g levels for 4 days.



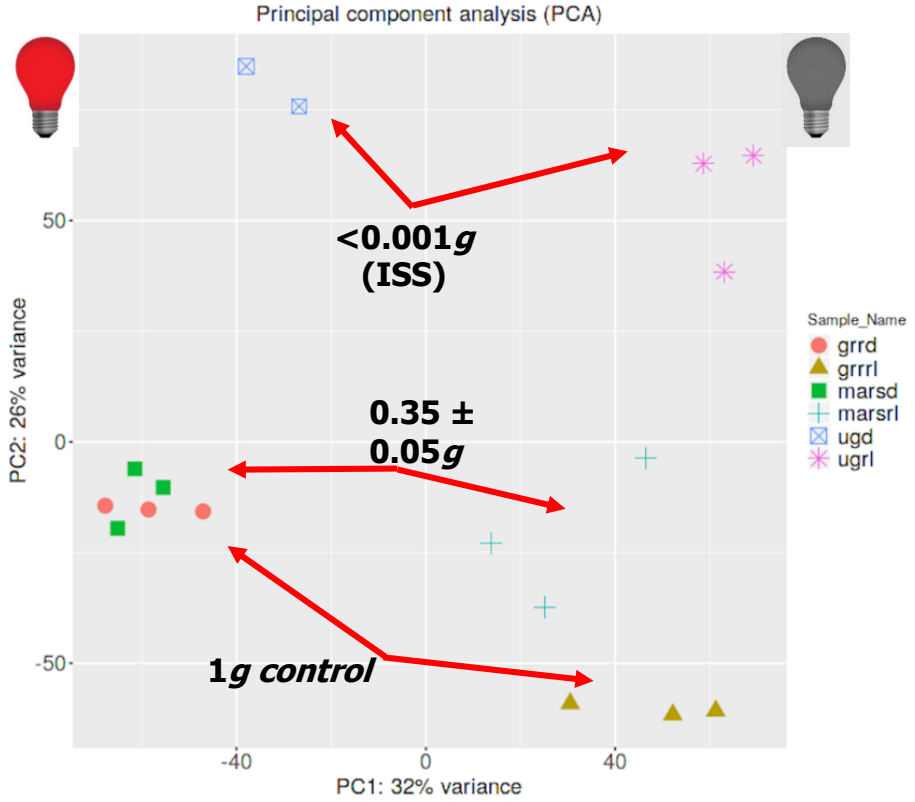




Manzano et al. *NPJ microgravity. (4)* 2018*)

Nuc1
Nuc2
Col0

Red photostimulation minimize transcriptome responses observed between 1g and Mars gravity

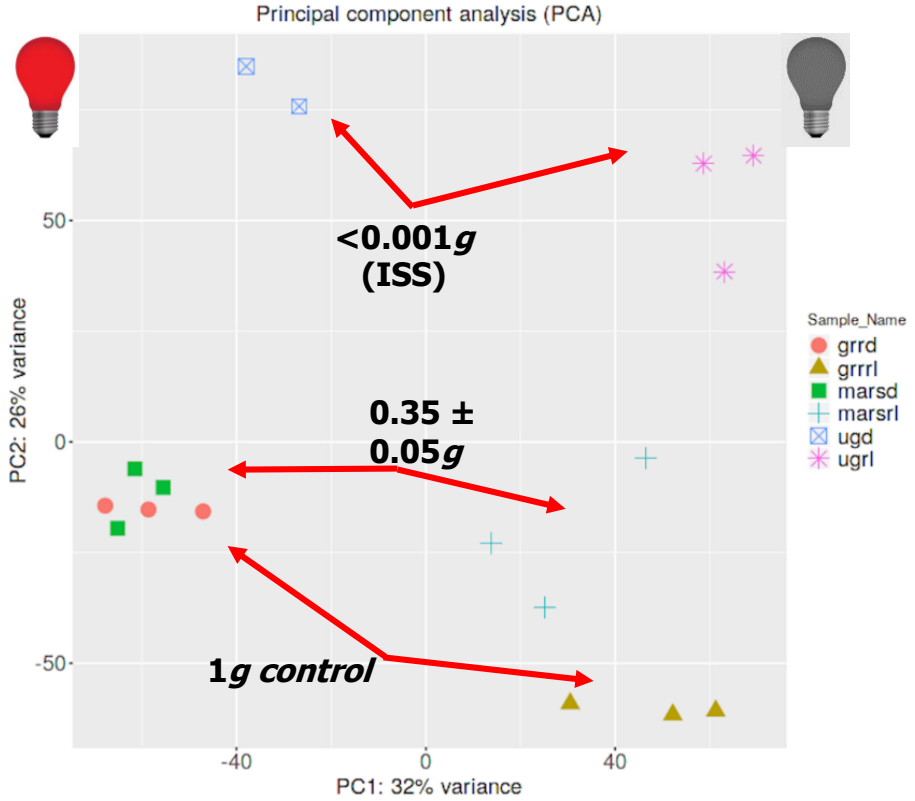


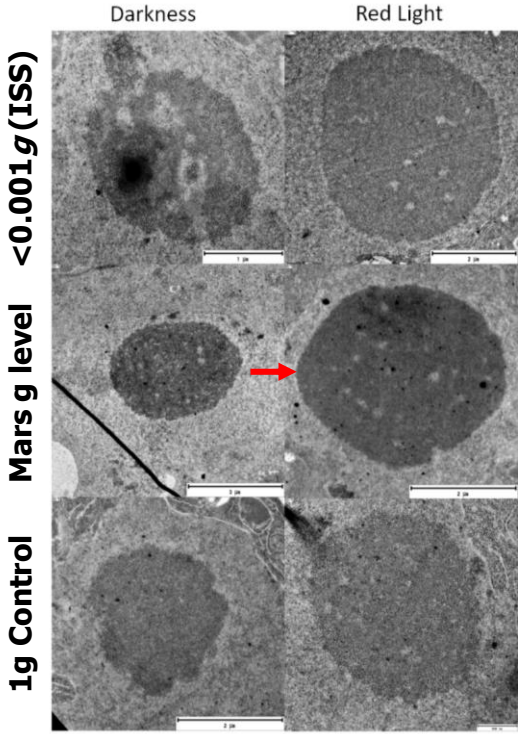
International Journal of *Molecular Sciences*

Villacampa et al., *International journal of molecular sciences 22 (2), 899,* **2021**

Nuc1
Nuc2
Col0

Red photostimulation minimize also the cell nucleolus level effects (stress sensor)





International Journal of *Molecular Sciences*

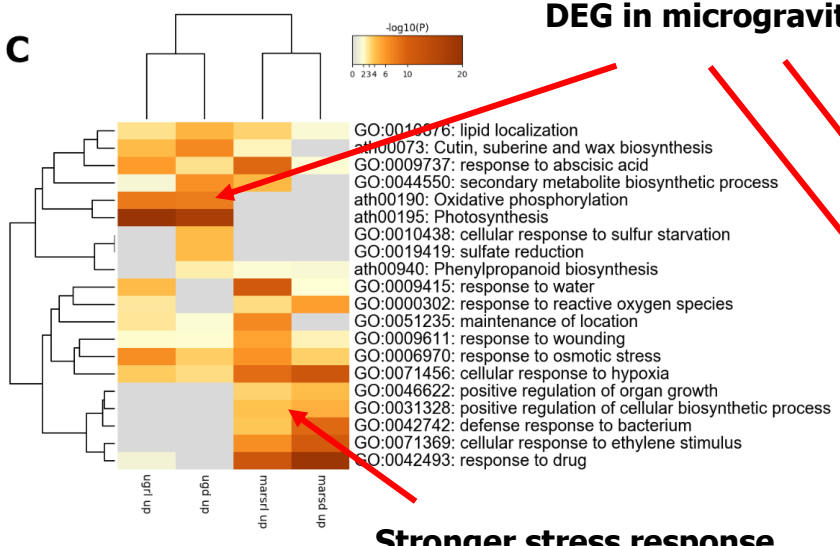
Villacampa et al., *International journal of molecular sciences 22 (2), 899,* **2021**

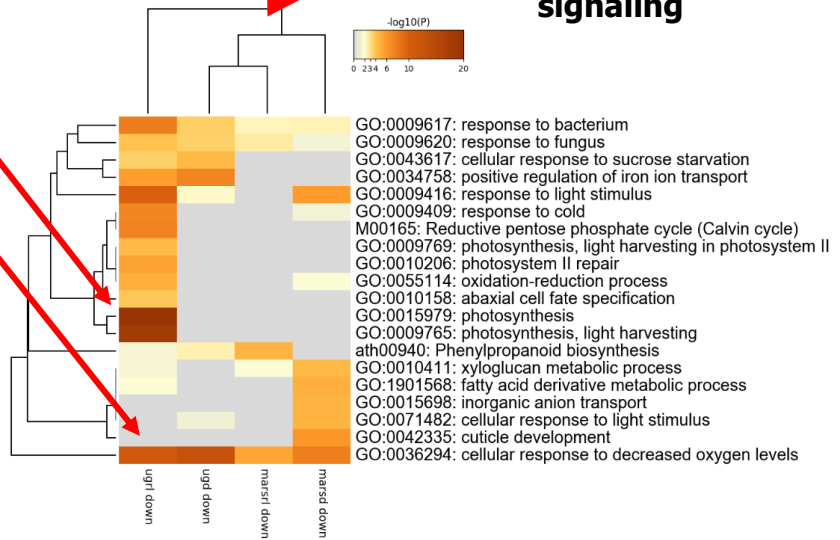
Nuc1
Nuc2
Col0

Complex response to partial g on the ISS is also observed in red photostimulated plants

Hypoxia, Photosynthesis and Oxidative stress are DEG in microgravity

Possible role of plastids/ Mitochondria retrograde signaling





Stronger stress response in partial gravity

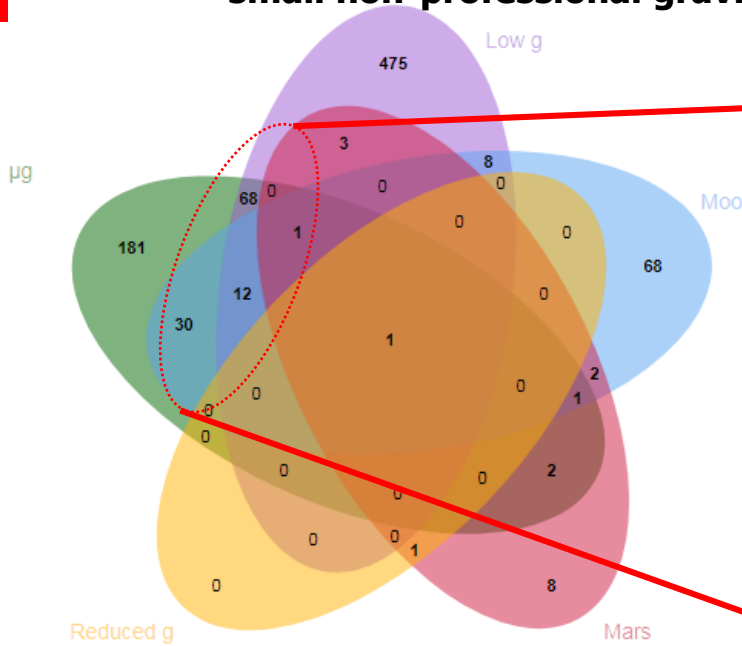
Alternative plant growth and stress related pathways are differentially activated

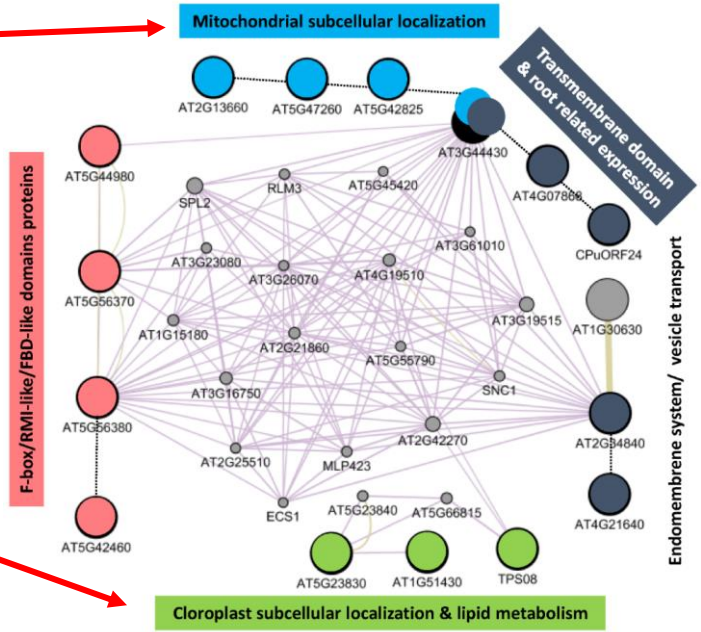
MDPI
International Journal of *Molecular Sciences*
Villacampa et al., *International journal of molecular sciences 22 (2), 899,* **2021**

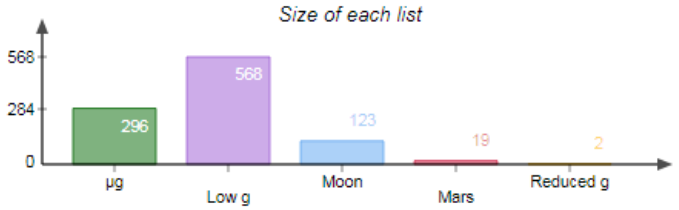
PhyA
PhyB
Ler

Very few DEGs are shared across reduced g-levels…

…but they can be connected in a relatively small non-professional graviresponse network





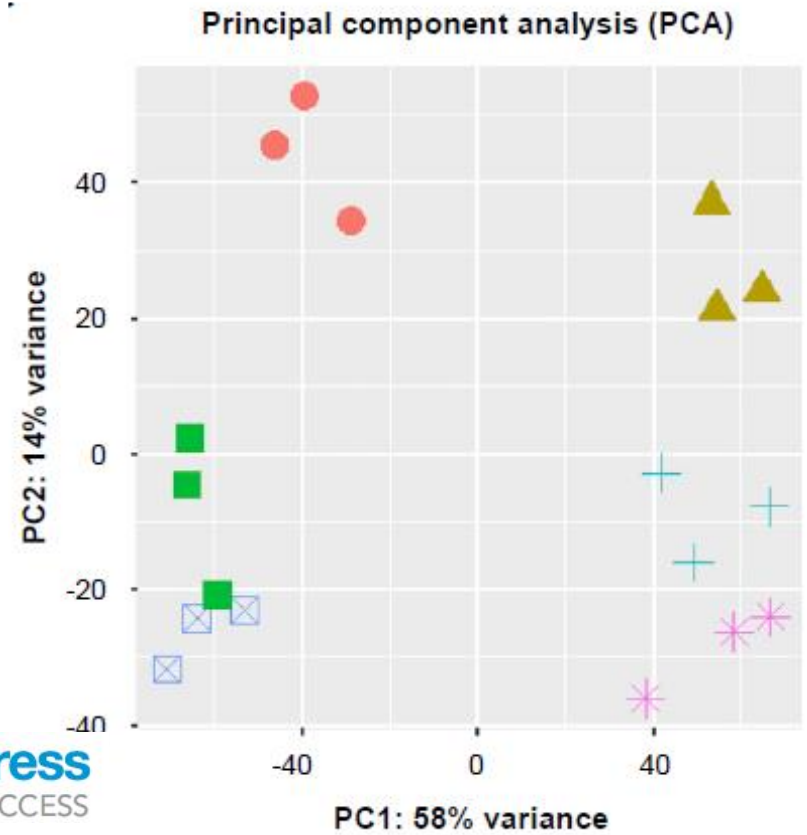


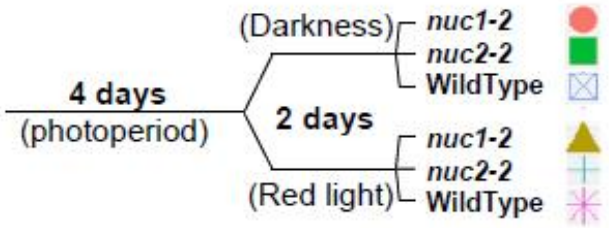
DEG (p<0.05 with FDR vs 1g control)

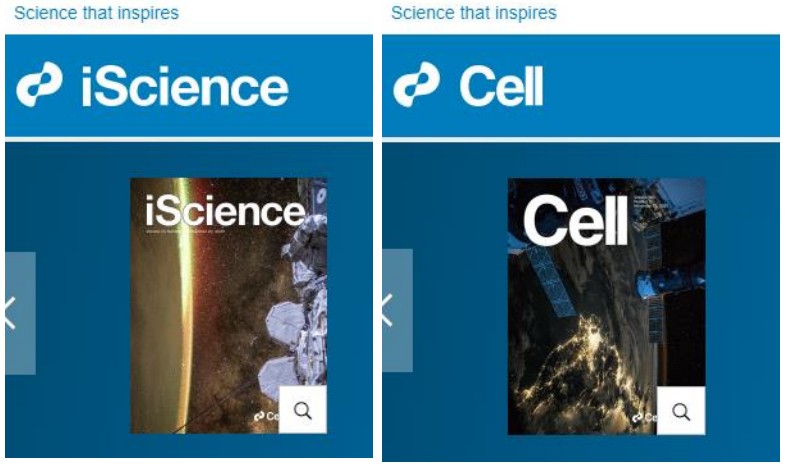
frontiers in Plant Science **Herranz et al. Frontiers in Plant Science 2019. DOI 10.3389/fpls.2019.01529**

Nuc1
Nuc2
Col0

Validating the transcriptome variations in the mutants in Ground reference tests







https://www.cell.com/c/the-biology-of-spaceflight

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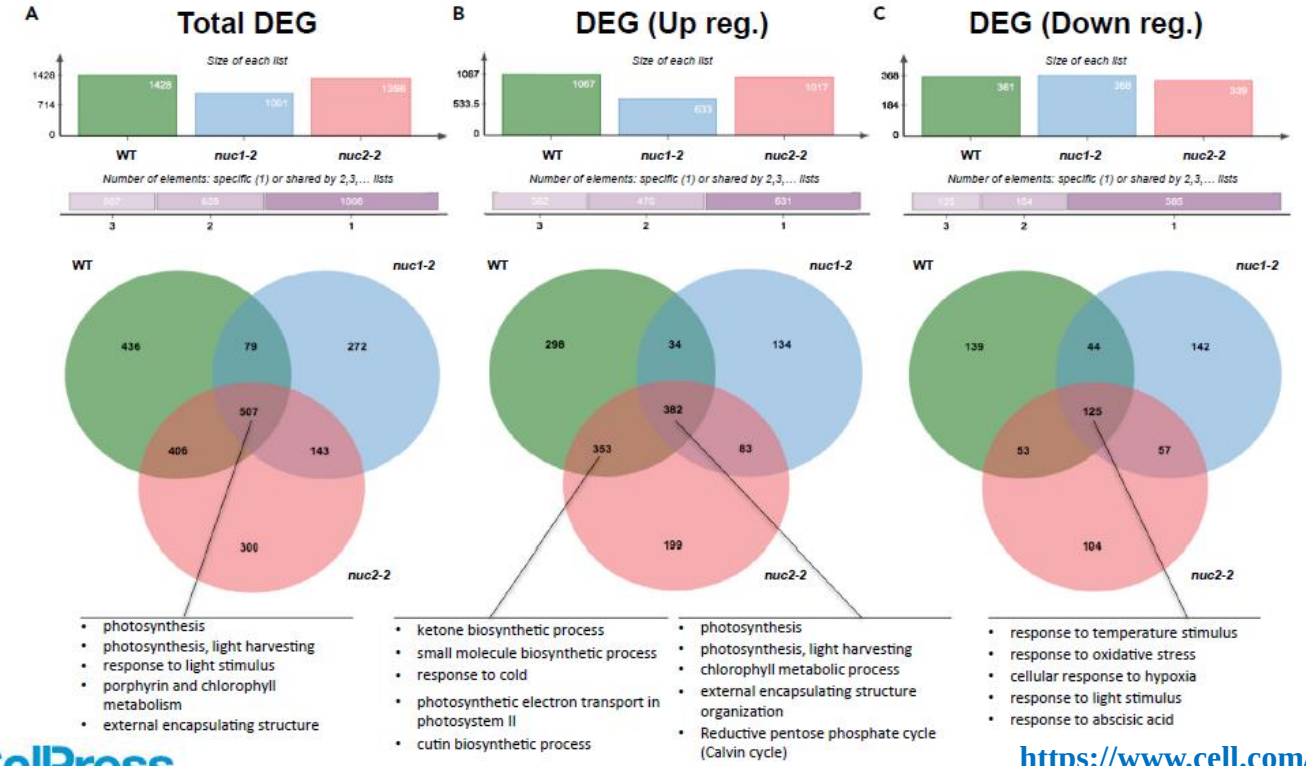
Manzano et al., iScience 23, 101686. Nov 20th **2020**

Nuc1
Nuc2
Col0

Nucleolin mutants show differential transcriptional adaptions under Red light and Darkness conditions



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Space Omics
An esa funded Topical Team

Nuc1
Nuc2
Col0

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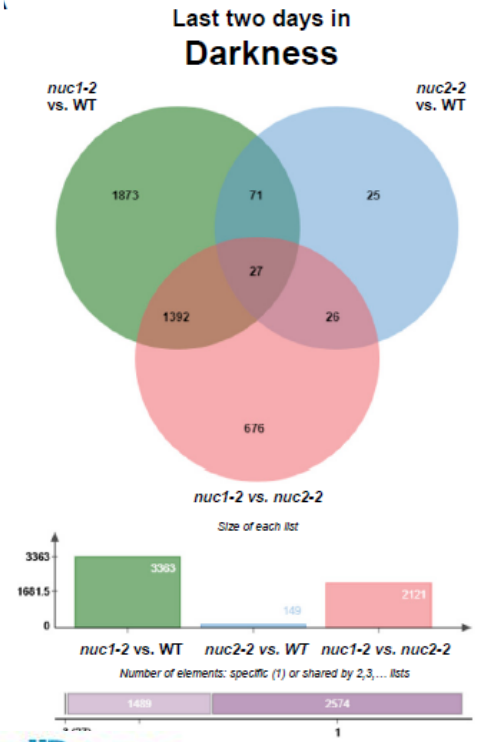

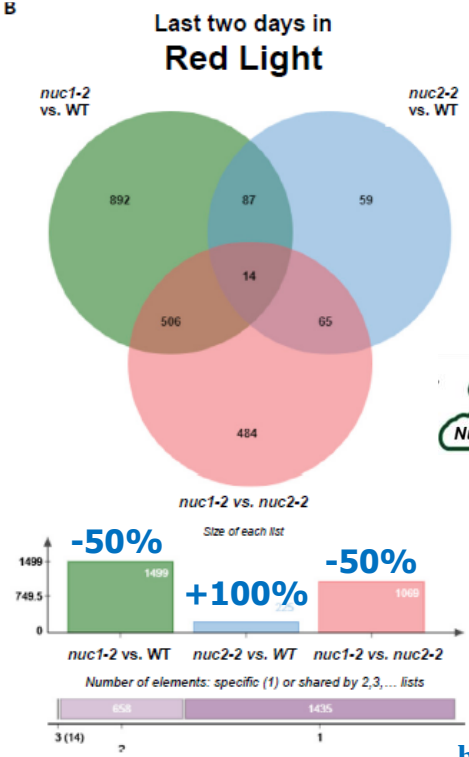

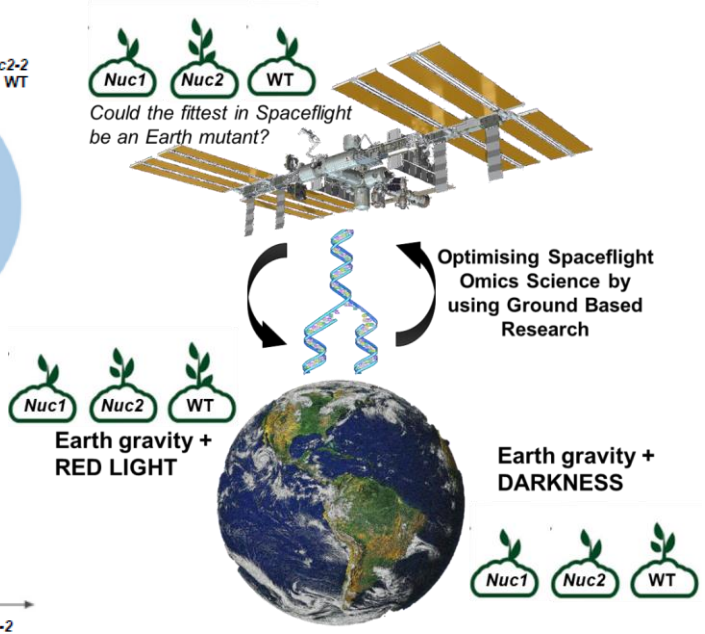

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Preliminary RNA-seq results on the nucleolin collection

PhyA
PhyB
Ler

36 Samples (6 conditions x 3-4 replicates)

BLUE LEDs **x** ***µg (ISS) <0.1g Moon g Mars g .57g 1g (ISS)*** **x** ***PhyA PhyB***

GLDS 346

Nuc1
Nuc2
Col0

62 Samples (8 conditions x 3 mutants x 2-3 replicates)

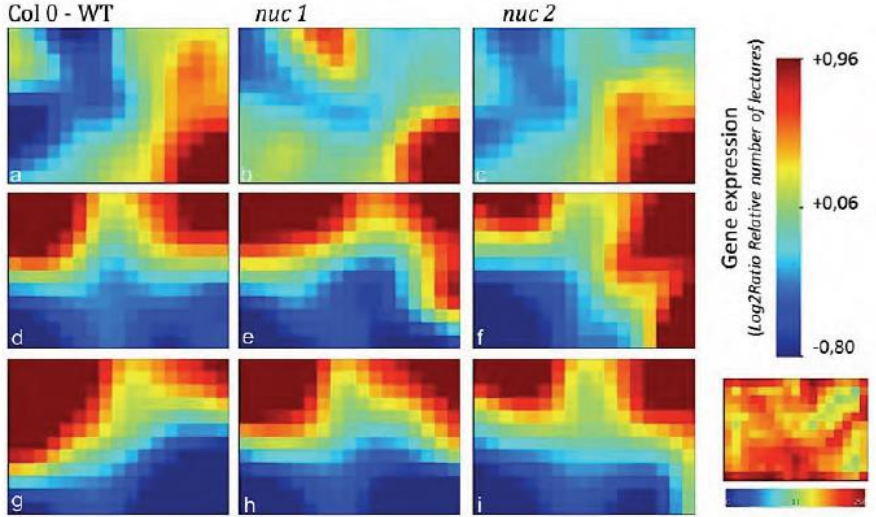
DARKNESS RED LEDs **x** ***µg (ISS) 0.3g (ISS) 1g (ISS) 1g (GRT)*** **x** ***Wt (Col0) Nuc-1 Nuc-2***

GLDS TBD

Microgravity effects (with Red light)
(Red -µg VS Red -1g)

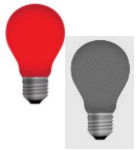
Red light effects (in microgravity)
Red -µg)

Microgravity effects (in darkness)
(Dark -µg VS Red -1g)



Preliminary RNA-seq results on the auxin signalling collection
Each mutant show a complementary response

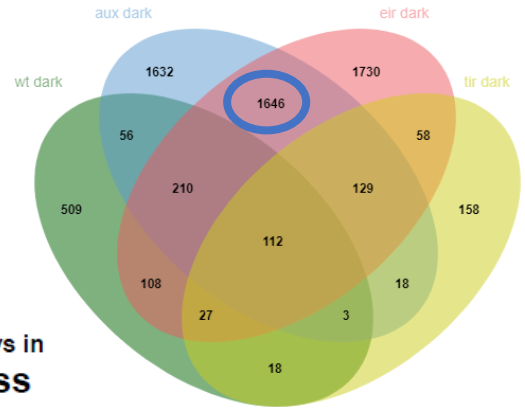
Aux1.7
Eir1.1
Tir1
Col0



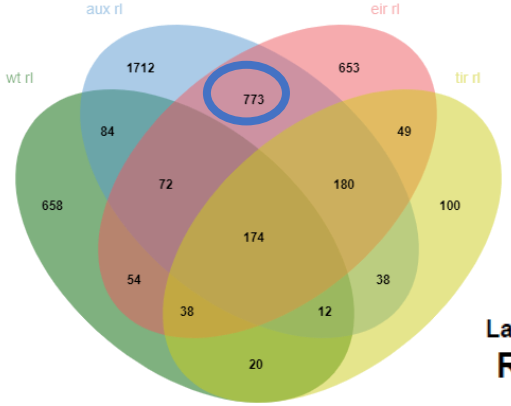
64 Samples (8 conditions x 4 lines x 2 replicates)

DARKNESS RED LEDs **x** ***µg (ISS) 0.3g (ISS) 1g (ISS) 1g (GRT)*** **x** ***Aux1.7 Eir1.1 Tir1 Col0***

GLDS TBD

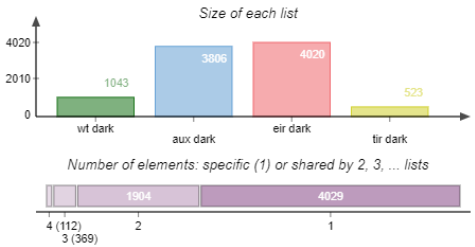


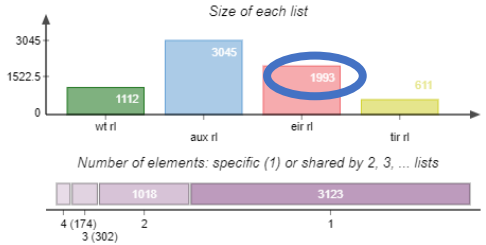
Last two days in
Darkness



Last two days in
Red Light

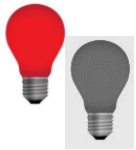
Aux response seems to be independent of ligth





Preliminary RNA-seq results on the auxin signalling collection
Each mutant show a complementary response

Aux1.7
Eir1.1
Tir1
Col0

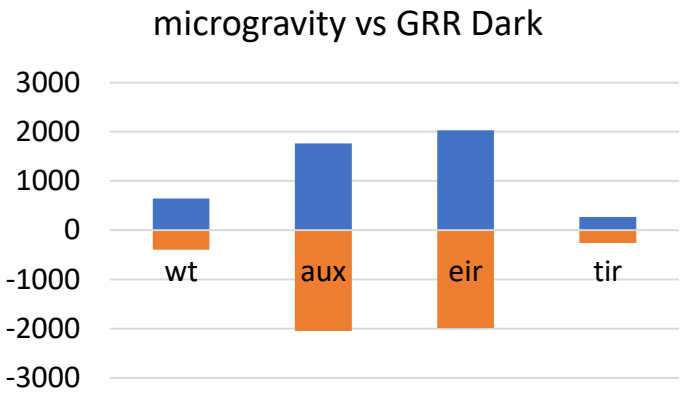


64 Samples (8 conditions x 4 lines x 2 replicates)

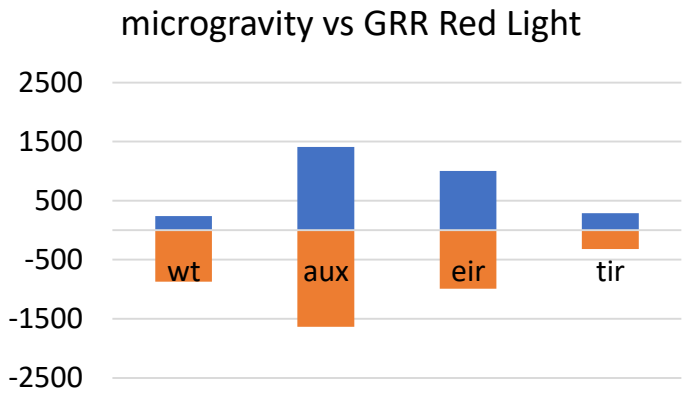
DARKNESS RED LEDs **x** ***µg (ISS) 0.3g (ISS) 1g (ISS) 1g (GRT)*** **x** ***Aux1.7 Eir1.1 Tir1 Col0***

GLDS TBD

Last two days in
Darkness



Last two days in
Red Light



Tir mutant is less sensible than WT,
Eir response is similar to WT only
when red light is present

Conclusions

PhyA
PhyB
Ler

In the presence of blue light photostimulation:

- Microgravity leads to alterations in phototropism and photosynthesis related genes, suggesting that phototropism may be compensating the lack of the gravitropism signaling
- Very low *g* levels (<0.1*g*) cause a severe and global stress response, not observed at the Moon *g* or the Micro*g* samples. A conflict between the weak blue phototropism (not observable at >.1*g*) and gravitropism signals may be triggering this acute response.
- At higher partial *g* conditions a weak response appears, inversely correlated with *g* level, mainly related with cell structure/organelles membranes suggesting this response may be linked to graviresistance or even an ancient retrograde signaling mechanism.
- *It should be reviewed that simulated microgravity experiments may be reflecting also this effect, overestimating transcriptional stress avoiding us to find the real microgravity response.*

Nuc1
Nuc2
Col0

In the presence of red light photostimulation:

- Wt results confirm blue light effect, differential response in each g level not only in quantity but in the type of GO and pathways affected to cope with the novel reduced *g* environment
- Global response to microgravity is also depending on the genetic background (stress genes mutants, as *nuc2*, may be more resilient avoiding over-responses to unknown stresses).
- Response to altered gravity conditions is also less important in mutants with reduced growth in Earth gravity (as *nuc1 or eir1*).
- Ongoing analyses on the complete collection of mutants from SG will help us validate the Seedling Growth wildtype samples results and simulated microgravity studies performed so far.

Aux1.7
Eir1.1
Tir1
Col0

FUNDING

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ROOTROOPS GBF/ESA
UNOOSA ZGIP

More info at these webpages:

https://issop.space/space-omics-topical-team

https://www.cell.com/c/the-biology-of-spaceflight

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THANK YOU.

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