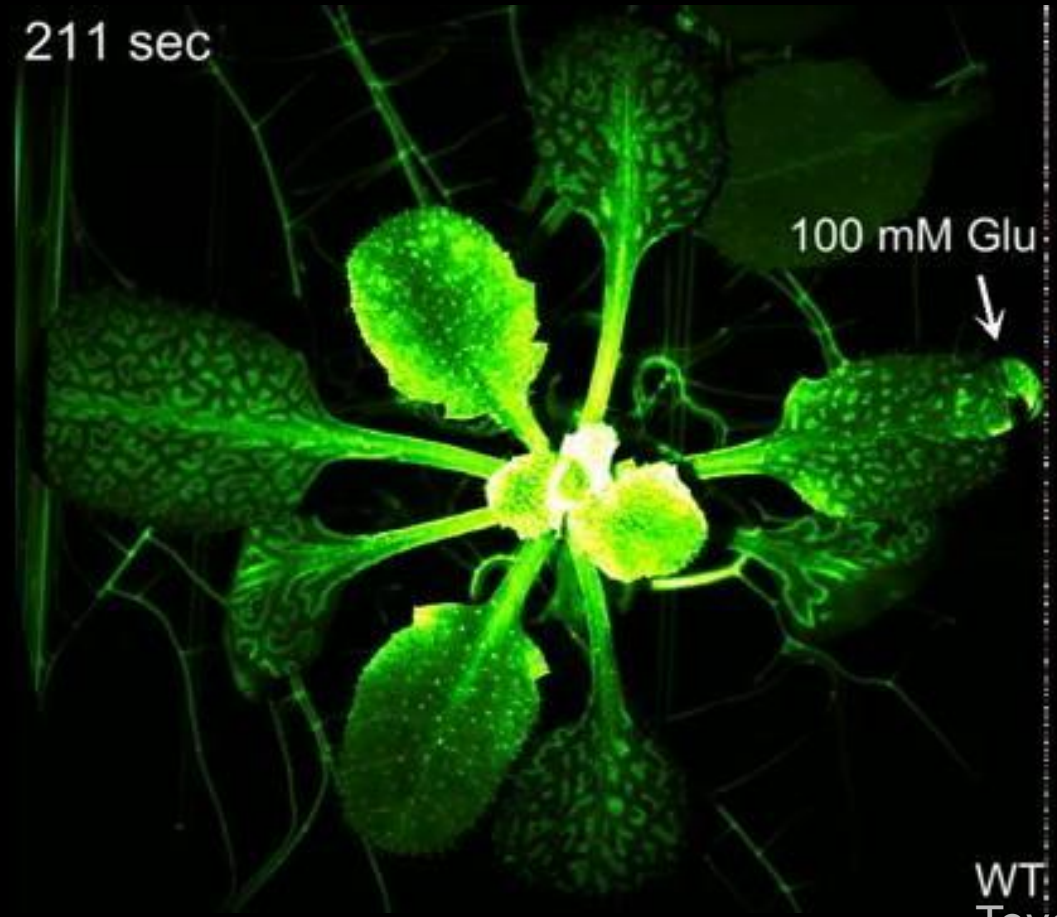
Time-lapse microscopy (Gcamp calcium sensor)

WISCONSIN
UNIVERSITY OF WISCONSIN-MADISON

Wild Type



Toyota et al., (2018)

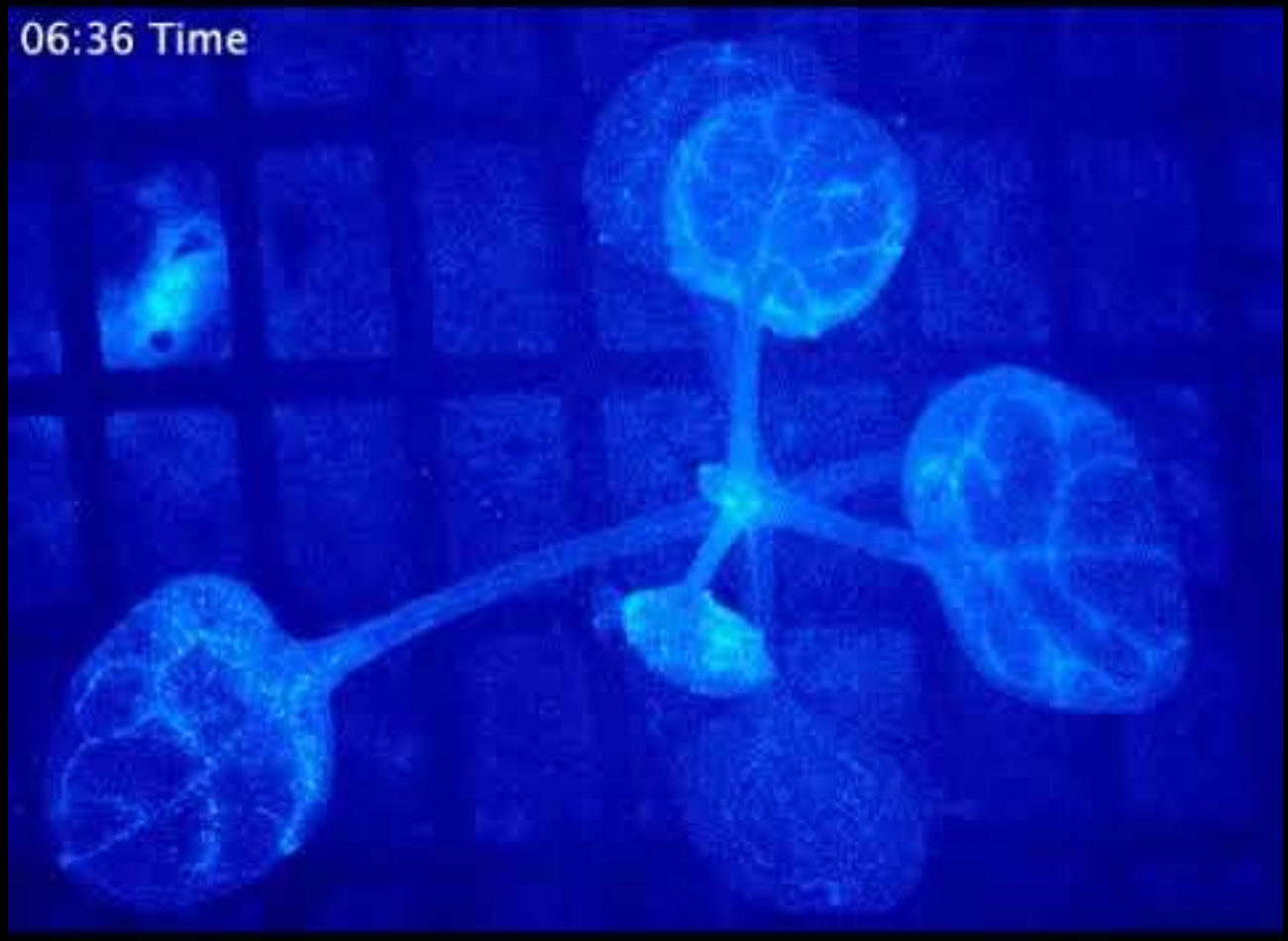
Example stimuli: Root flood, wild type images

WISCONSIN
UNIVERSITY OF WISCONSIN-MADISON

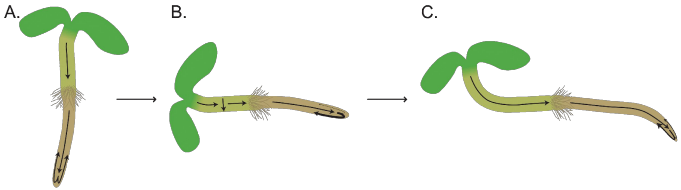
Wild Type

Flooded
root system

GCamp



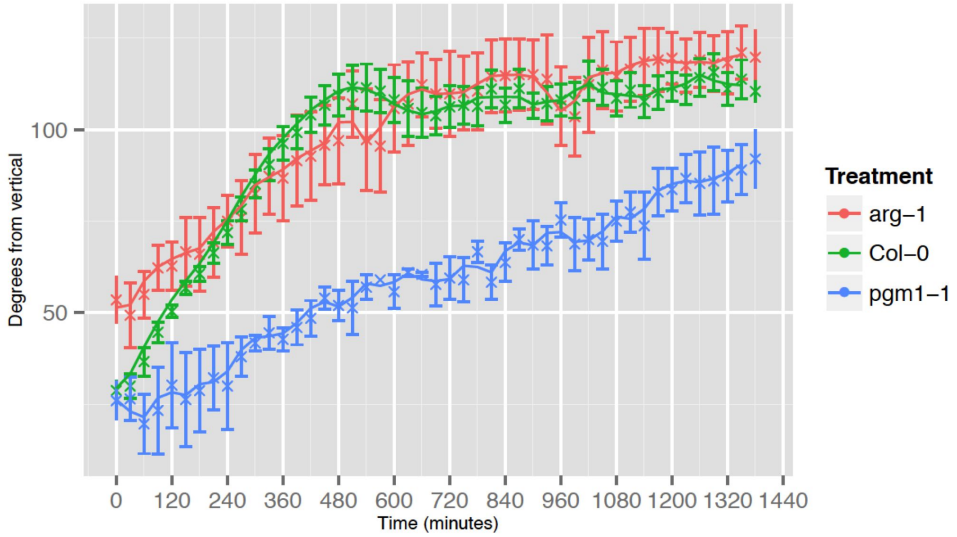
I moved to the US to study gravity sensitivity



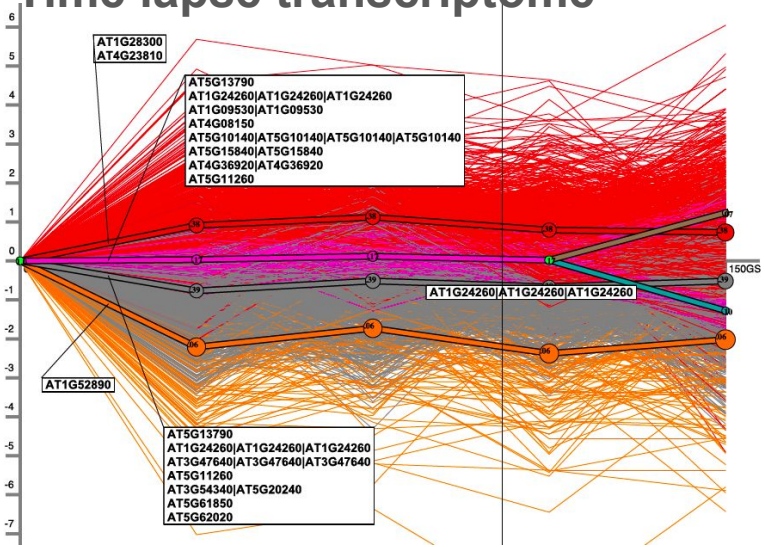
Masson Lab

APEX06

Time lapse morphology



Time lapse transcriptome



Outreach event

WARF

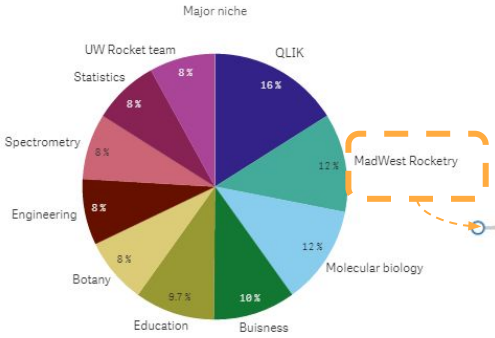
Wisconsin Alumni Research Foundation

So many genees, I needed help (& a robotic photography rig)!

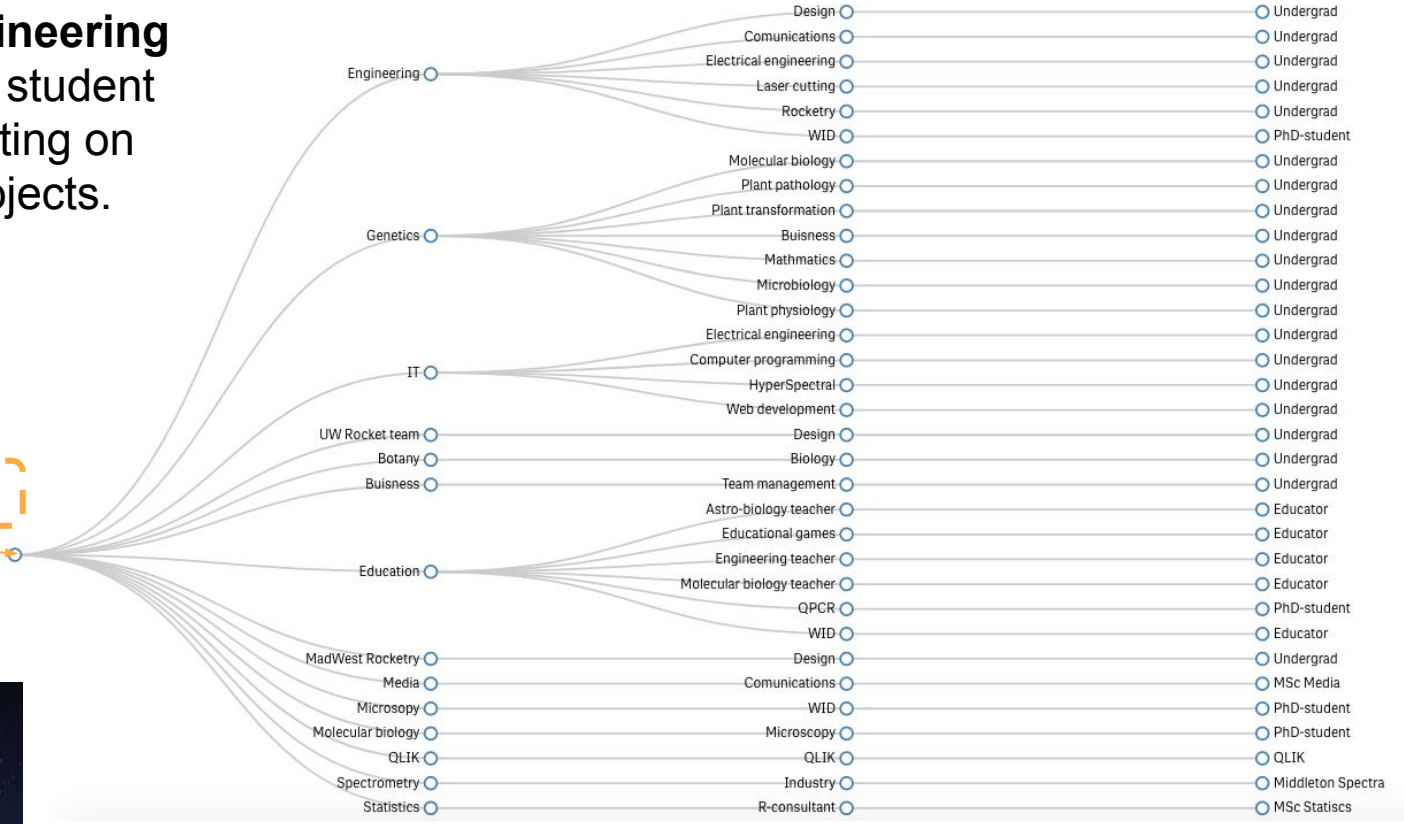
So we started a series of students organisations eg “FlashLapse”.

Supporting your local science club and they will support you.

Astro-Botanical Engineering Course: coordinated student volunteers collaborating on interdisciplinary projects.







& We applied to NASA for flight mission… fingers crossed!

The MadWest High School Rocket team started preparing for a launch of their own!

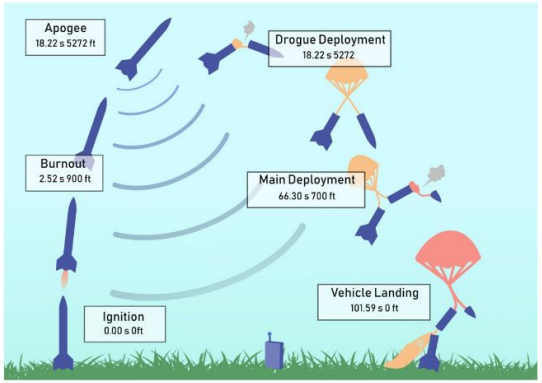


Figure 9: Mission profile chart

The table below summarizes the flight events for the entire mission.

Sequence	Event	Time	Altitude	Trigger
1	Ignition/Boost	0.00 s	0 ft	Launch Control
2	Burnout	2.52 s	900 ft	--
3	Apogee	18.22 s	5272 ft	--
4	Drogue deployment	18.22 s	5272 ft	Altimeter
5	Main deployment	66.30 s	700 ft	Altimeter
6	Vehicle Landing	101.59 s	0 ft	--



As they completed their flight Readiness Review we discussed what effect it might have on the gravity sensing cells in the Columella?

Prepping Payload/Rocket T-24 hours



This time they had a precious payload to take care off.

Plant Care for Rocket Fair





They knew how to care for the plants and codes.









Successful Launch!
Welcome to the Mile high club (+/-1m).

Payload Recovery

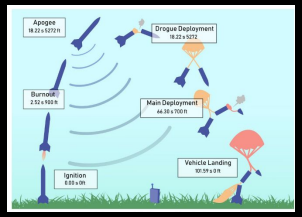






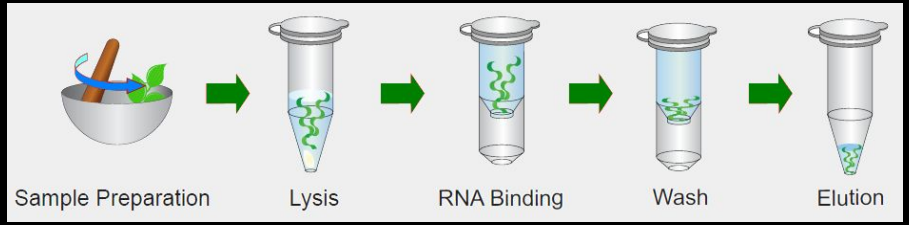
Payload Fixation





Samples were washed with PBS buffer and then RNA extracted from the QIAGEN RNAeasy kit

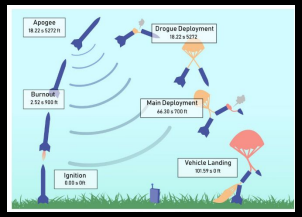
A Nanodrop was used to assess RNA quality and quantity





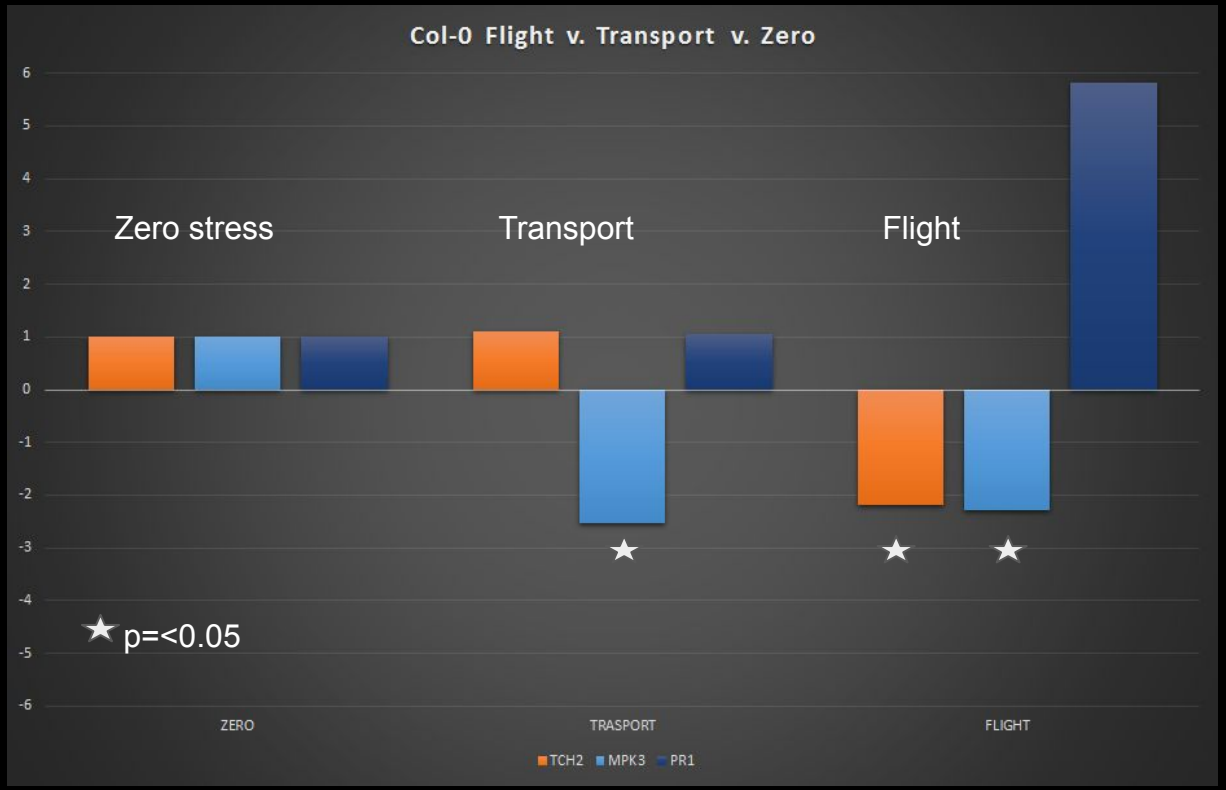
Enough RNA was extracted for Quantitative Polymerase Chain Reactions (QPCR) to be performed to quantify the expression of target genes.

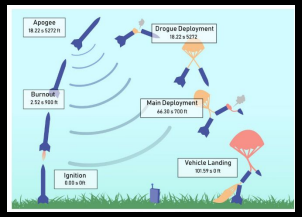
#	Sample ID	User name	Date and Time	Nucleic Acid Conc.	Unit	A260	A280	260/280	260/230	Sample Type
1		Gilroy Lab	4/19/2017 3:23:09 PM	31.3	ng/µl	0.783	0.494	1.59	0.64	RNA
2		Gilroy Lab	4/19/2017 3:23:56 PM	40.7	ng/µl	1.017	0.578	1.76	0.46	RNA
3		Gilroy Lab	4/19/2017 3:24:22 PM	30.0	ng/µl	0.750	0.435	1.72	0.15	RNA
4		Gilroy Lab	4/19/2017 3:24:43 PM	39.8	ng/µl	0.996	0.518	1.92	1.05	RNA
5		Gilroy Lab	4/19/2017 3:25:07 PM	31.7	ng/µl	0.792	0.432	1.83	0.16	RNA
6		Gilroy Lab	4/19/2017 3:25:30 PM	41.9	ng/µl	1.048	0.570	1.84	1.24	RNA
7		Gilroy Lab	4/19/2017 3:25:53 PM	51.5	ng/µl	1.288	0.707	1.82	1.44	RNA
8		Gilroy Lab	4/19/2017 3:33:50 PM	35.5	ng/µl	0.887	0.445	1.99	0.12	RNA
9		Gilroy Lab	4/19/2017 3:34:21 PM	39.9	ng/µl	0.997	0.550	1.81	0.48	RNA
10		Gilroy Lab	4/19/2017 3:34:54 PM	59.3	ng/µl	1.484	0.779	1.90	1.33	RNA
11		Gilroy Lab	4/19/2017 3:35:23 PM	109.9	ng/µl	2.746	1.375	2.00	1.60	RNA
12		Gilroy Lab	4/19/2017 3:35:50 PM	47.1	ng/µl	1.178	0.643	1.83	0.21	RNA
13		Gilroy Lab	4/19/2017 3:36:30 PM	24.8	ng/µl	0.621	0.428	1.45	0.11	RNA
14		Gilroy Lab	4/19/2017 3:37:00 PM	82.5	ng/µl	2.062	1.005	2.05	1.40	RNA
15		Gilroy Lab	4/19/2017 3:37:28 PM	54.5	ng/µl	1.363	0.724	1.88	0.96	RNA



QPCR on mechanical sensitive genes TOUCH2, PATHOGEN RELATED 1 and MAP KINASE 3

- MPK3 expression was repressed by the mechanical stimulation provided by the transport treatment.
- TOUCH2 expression was repressed by the mechanical stimulation provided by the Rocket flight
- PATHOGEN RELATED 1 was induced by rocket flight.
- Conclusion: Transporting plants large distances and/or rocket flight can affect their pathogen tolerance





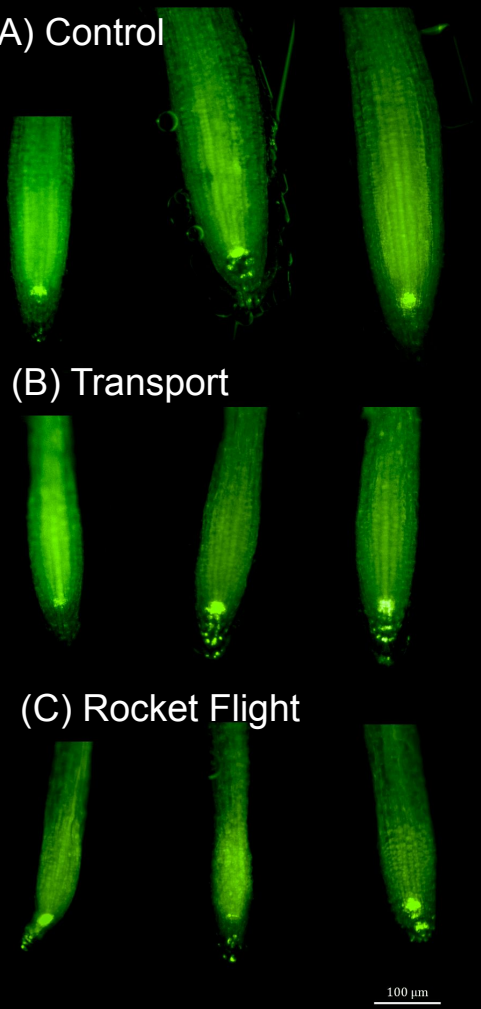
Rocket team conclusion: The mechanical stimuli caused by the transport and rocket flight affect auxin distribution within the gravity sensing cells of the root.

DRB conclusion: Further work and more replication is needed to be sure.

(A) Control

(B) Transport

(C) Rocket Flight

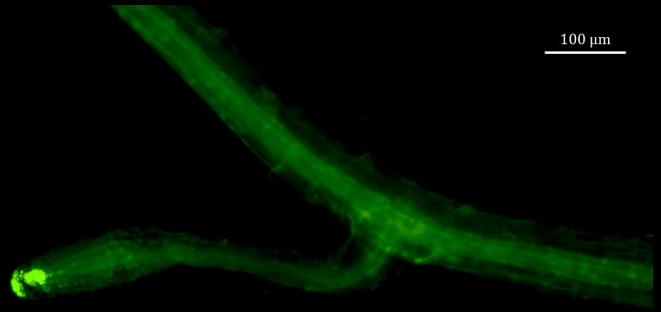


DR5:GFP-Venus can be used to observe auxin distribution

(A) The control zero stress group roots were generally larger

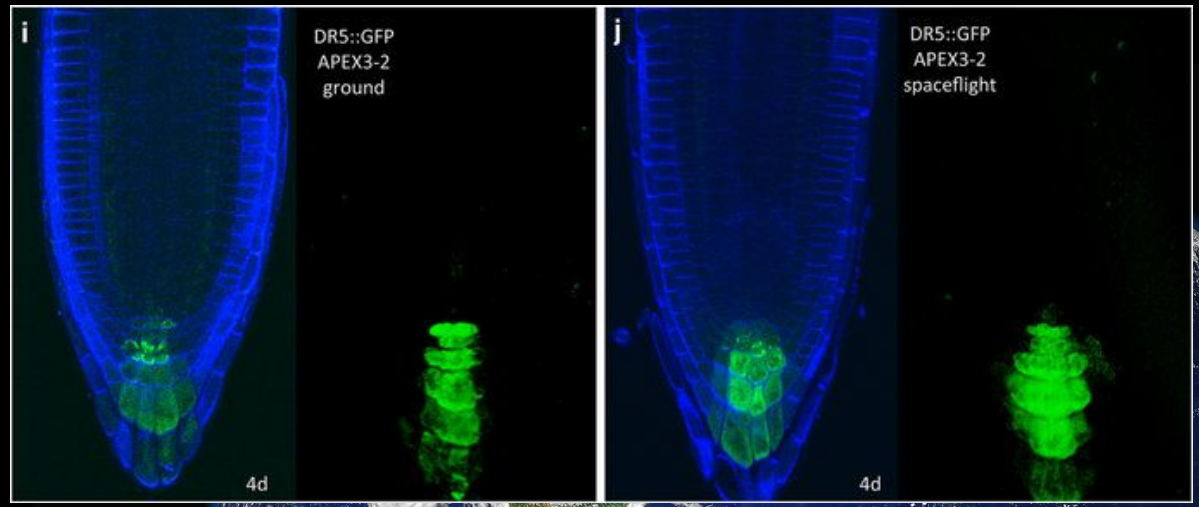
(B) Transport Stress group had small roots with more GFP in the S5 columella cells

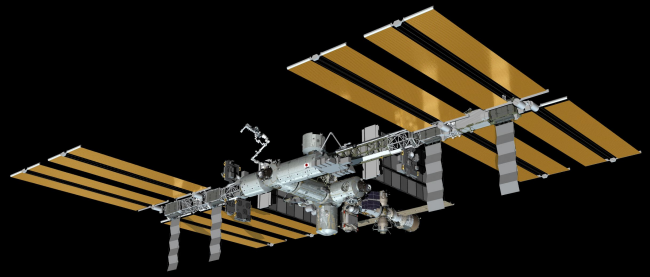
(C) Flight group had small roots with more GFP in the S4 columella cells



Ferl and Paul (2016) had recently published these images if DR5:GFP from plant on the International Space Station.

- Astronauts on the ISS observed subtle changes in auxin distribution.
- Both teams had difficulty getting enough replication.
- Exciting for the young rocket team





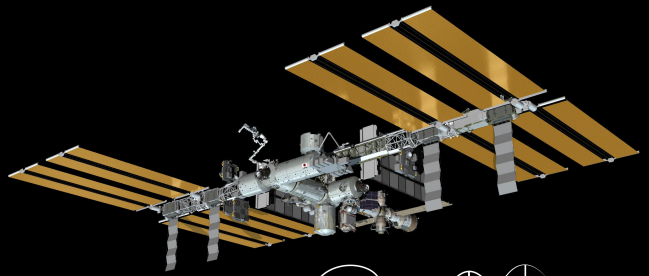


Then it was time for BRIC19 on SpaceX Dragon Crew Resupply mission 4 (CR-4)





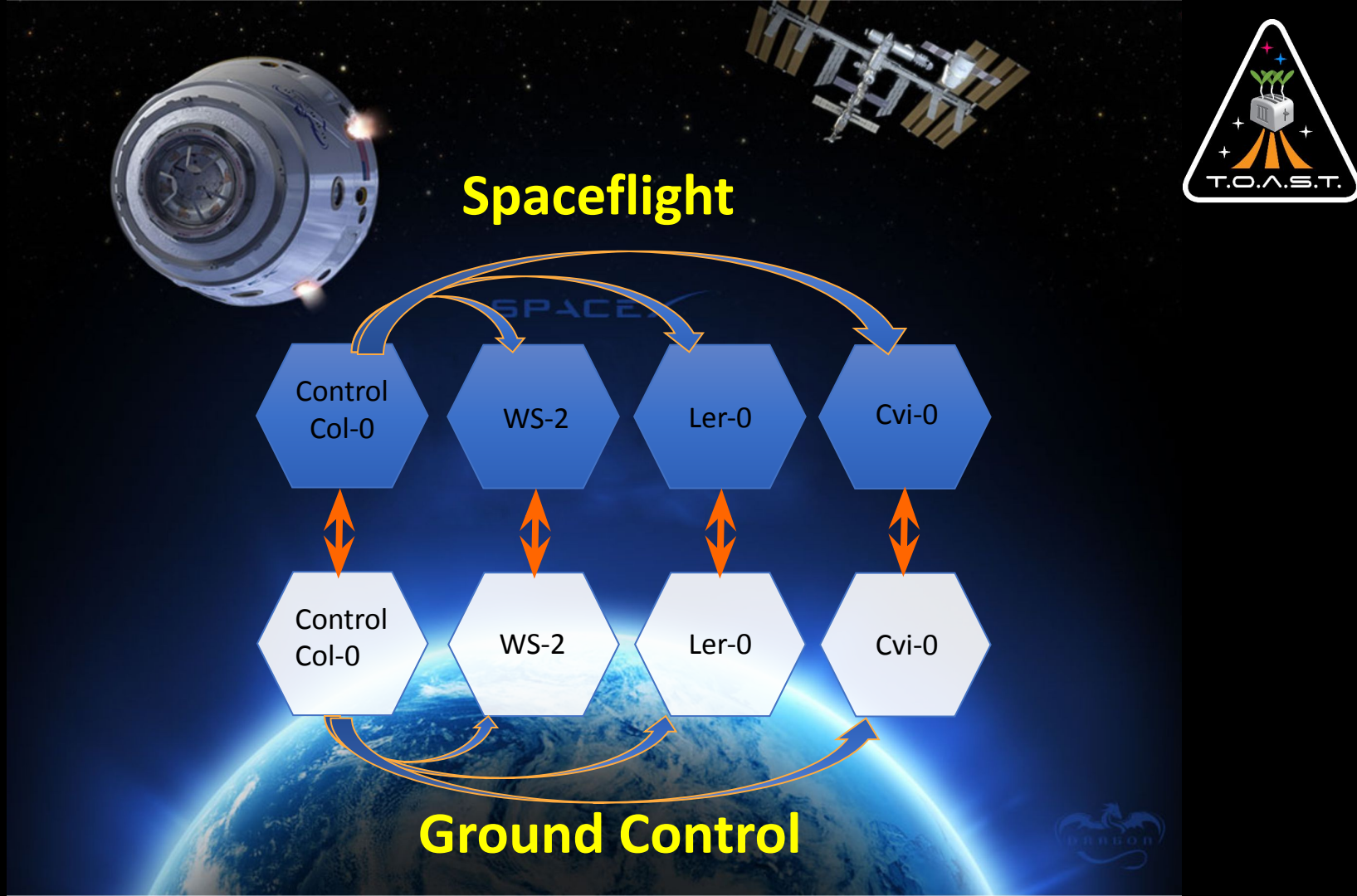
TOAST I: Test of Arabidopsis Space Transcriptome



GENE LAB



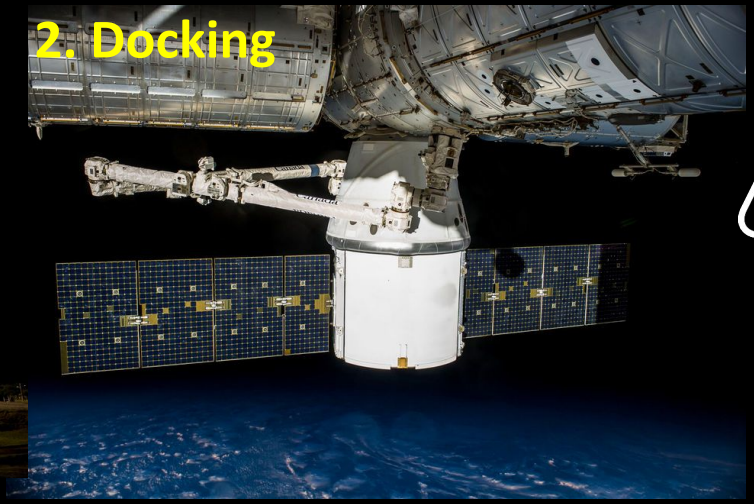
At this point GeneLab didn't have a logo.


Spaceflight
Control Col-0
WS-2
Ler-0
Cvi-0
Control Col-0
WS-2
Ler-0
Cvi-0
Ground Control
T.O.A.S.T.

1. Launch



2. Docking



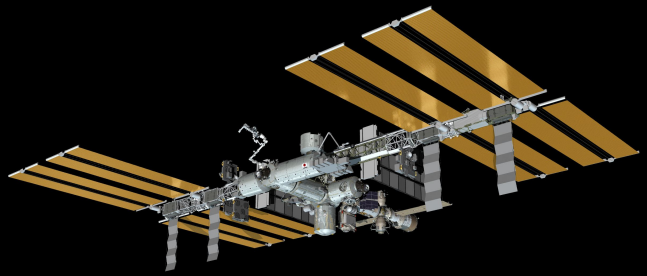
3. Sample fixation



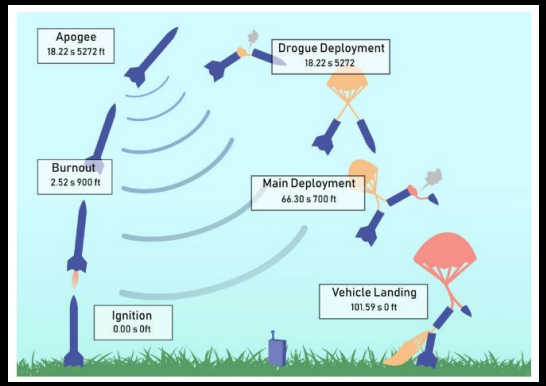
4. & return

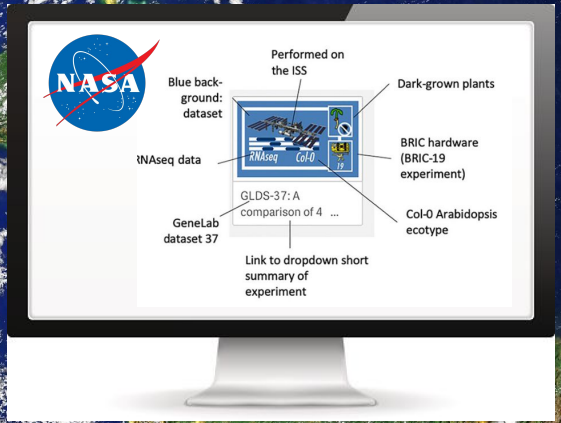


Then it was time for BRIC19 on SpaceX Dragon Crew Resupply mission 4 (CR-4)



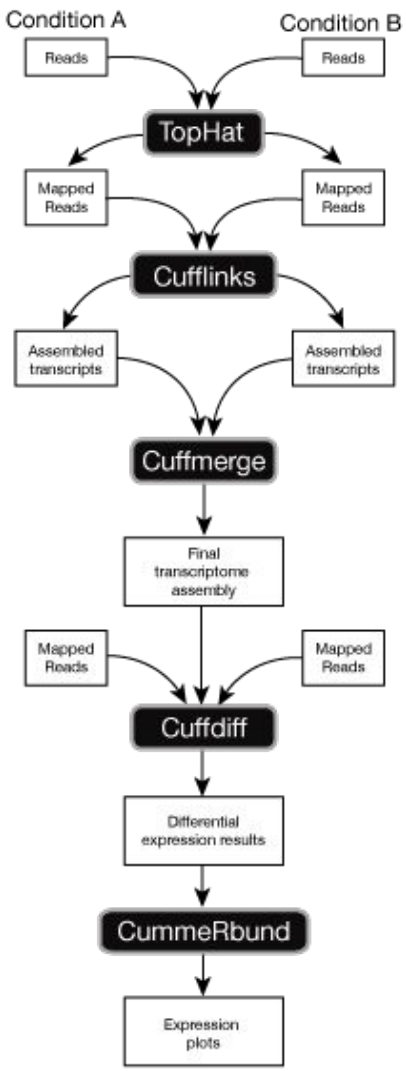






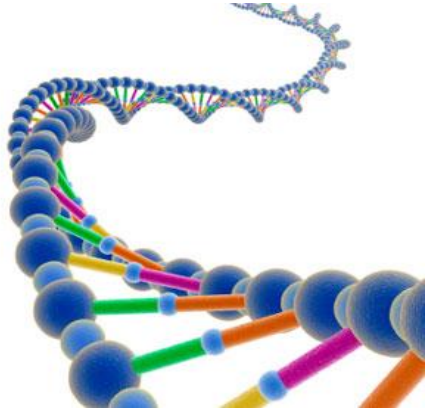


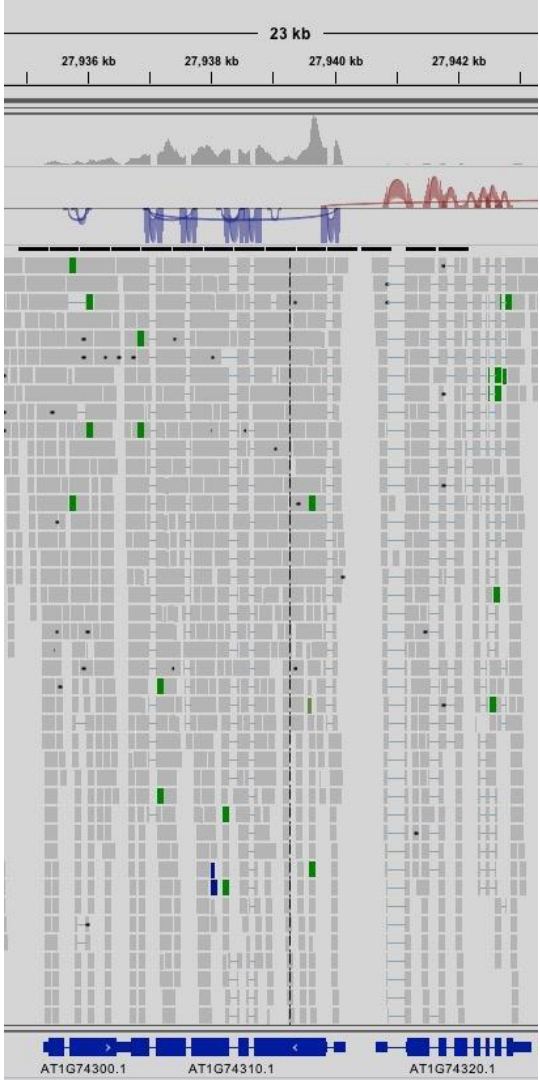
"Old school analysis"



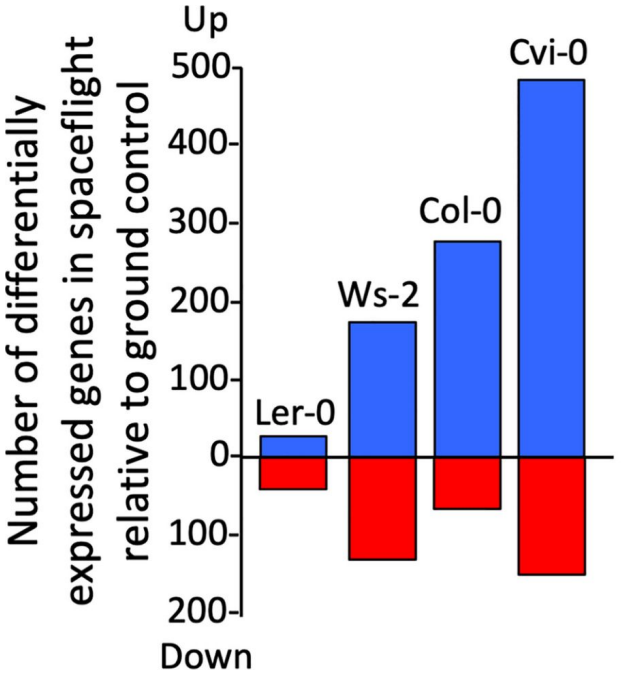
RNAseq

~80 million reads
56 samples
Paired end
100 bp fragments
33559 genes
41621 transcripts





3 methods identify genes affected by the spaceflight

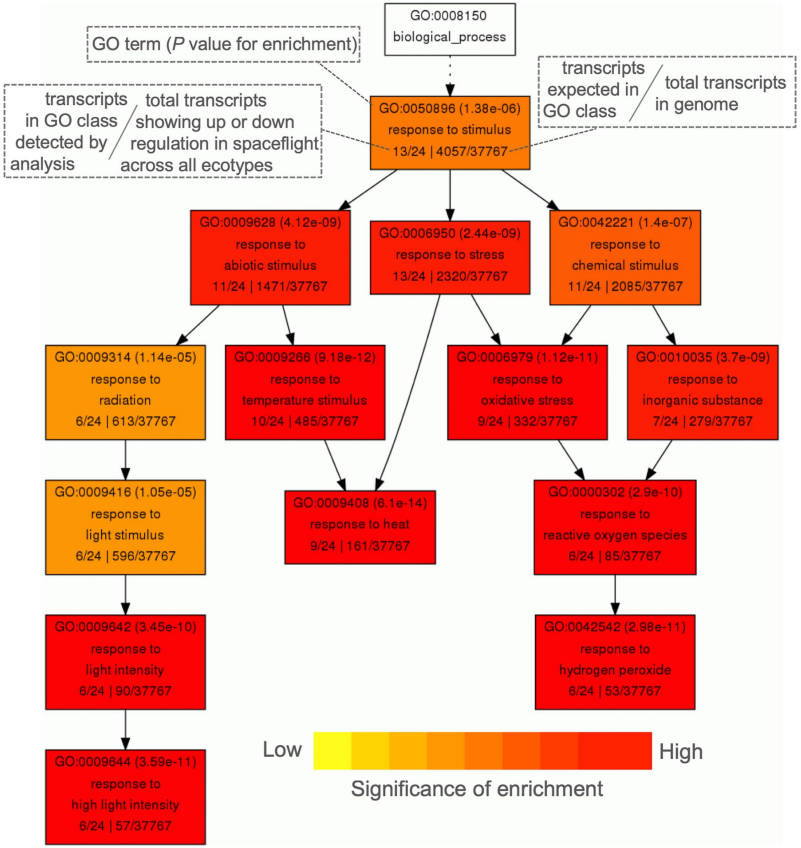




"Gene Prioritisation by evidence counting"

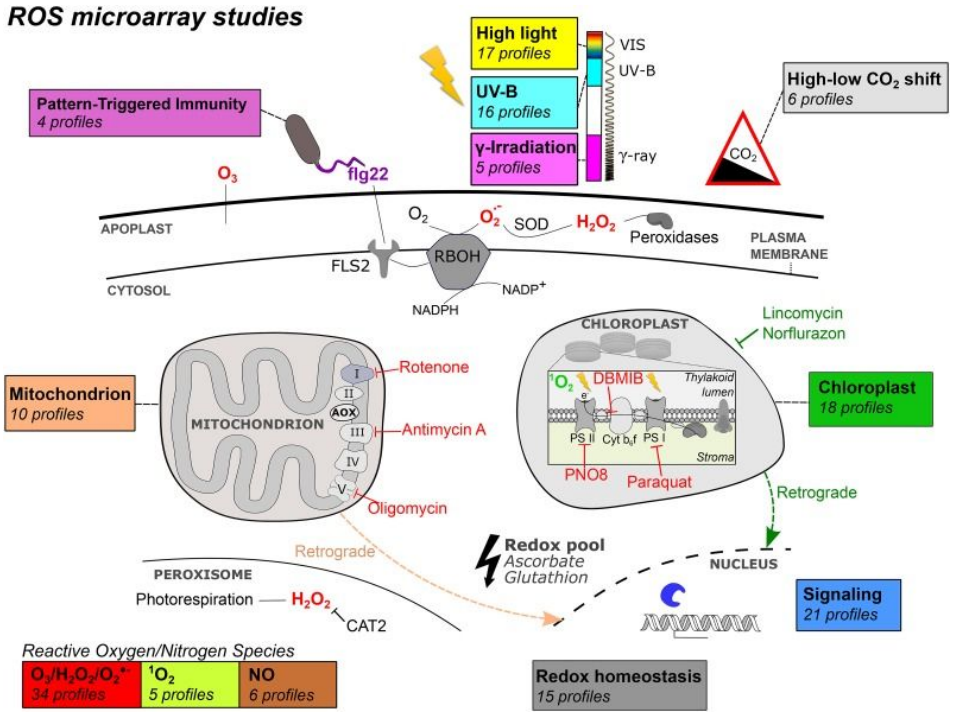
Choi et al, (2019)

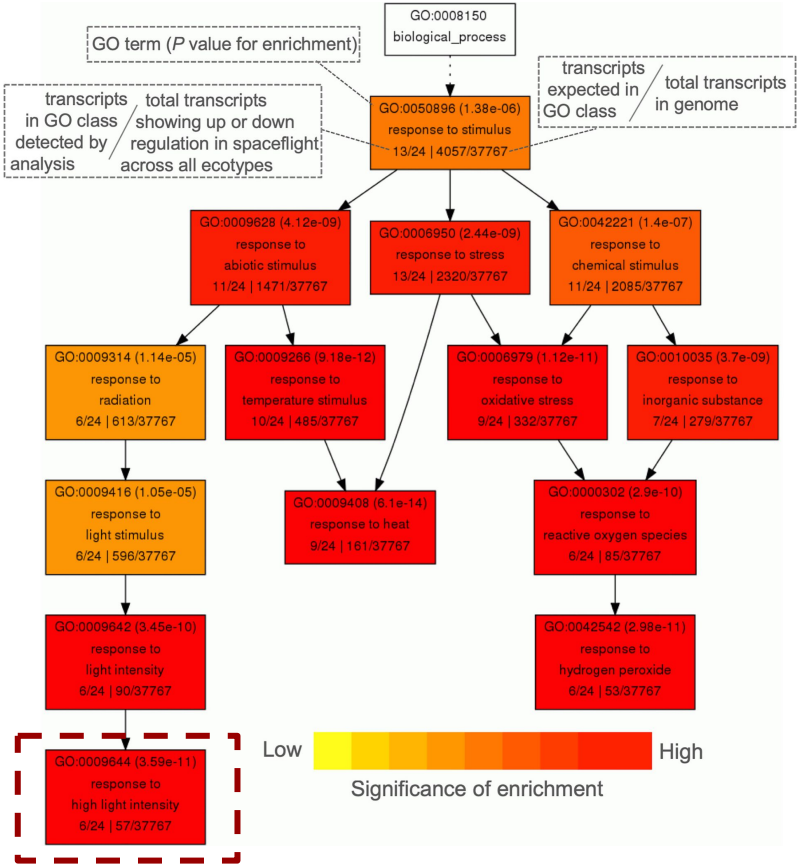
Spaceflight induced the “Highlight” response



Choi et al, (2019)

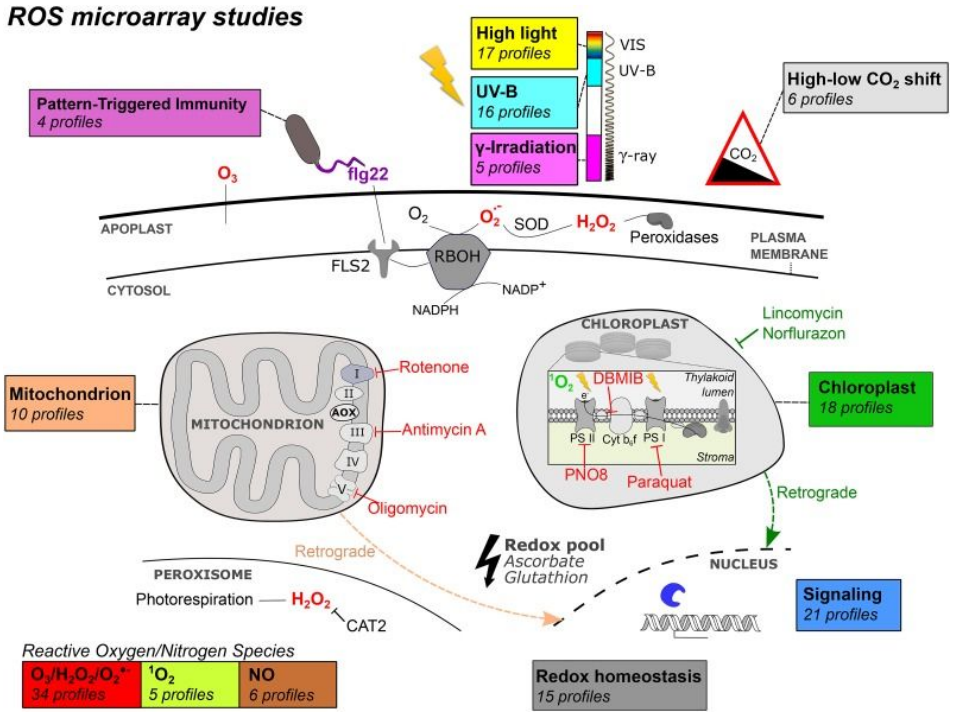
Many potential subcellular sources of ROS

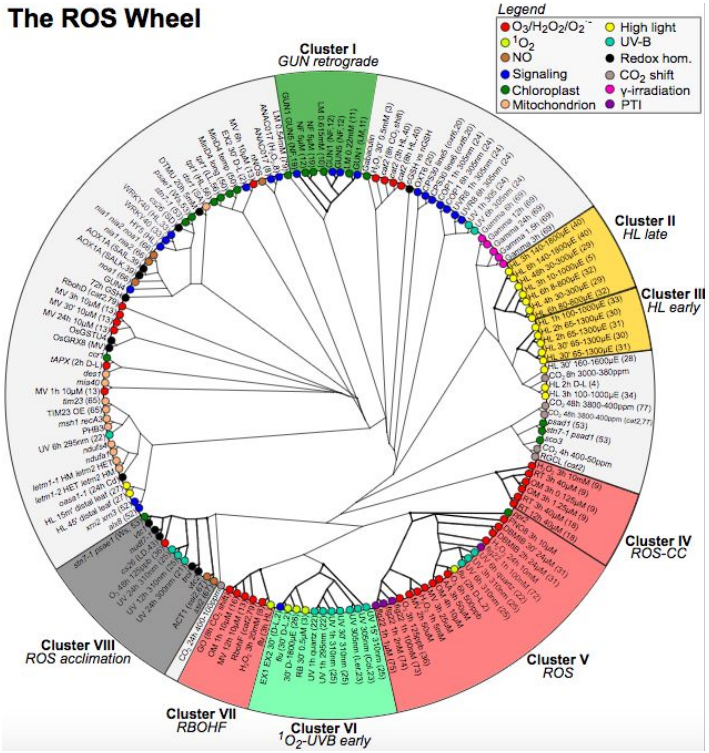




Willems et al, (2016)

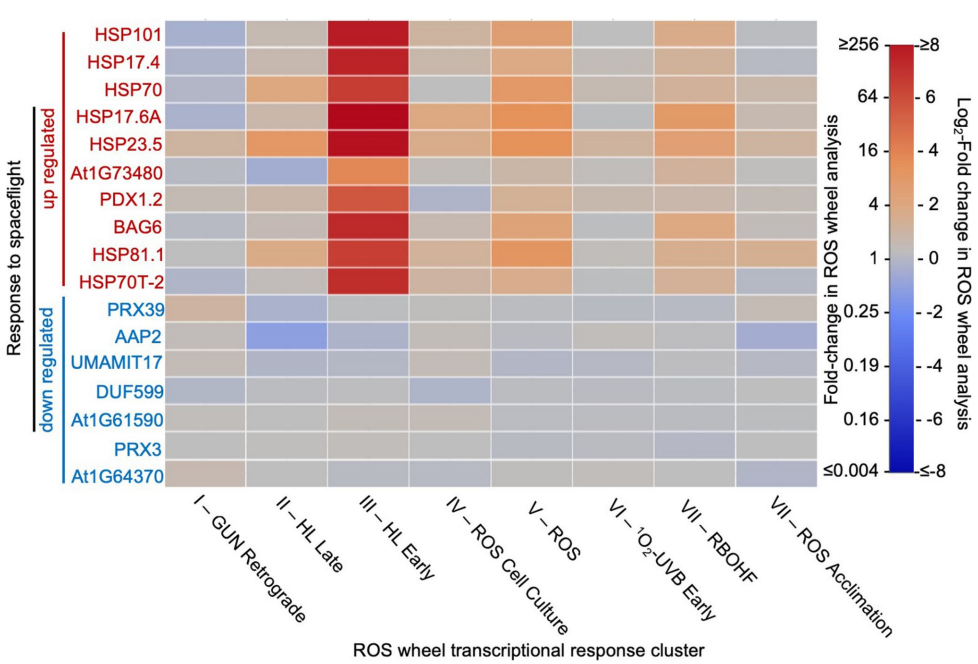
European Bioinformatic Expression Atlas reveals correlation with “high light”



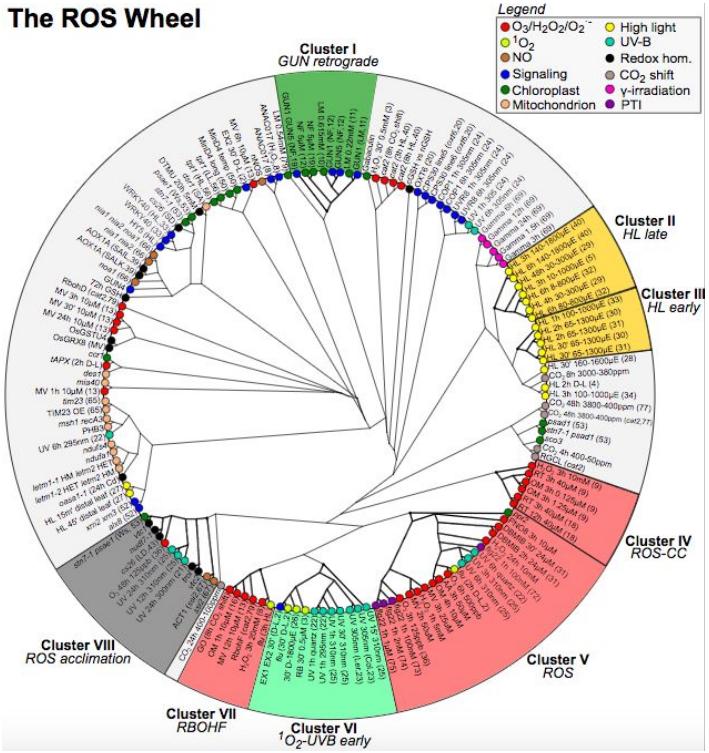


Willems et al, (2016)

Highlight response involves heatshock proteins

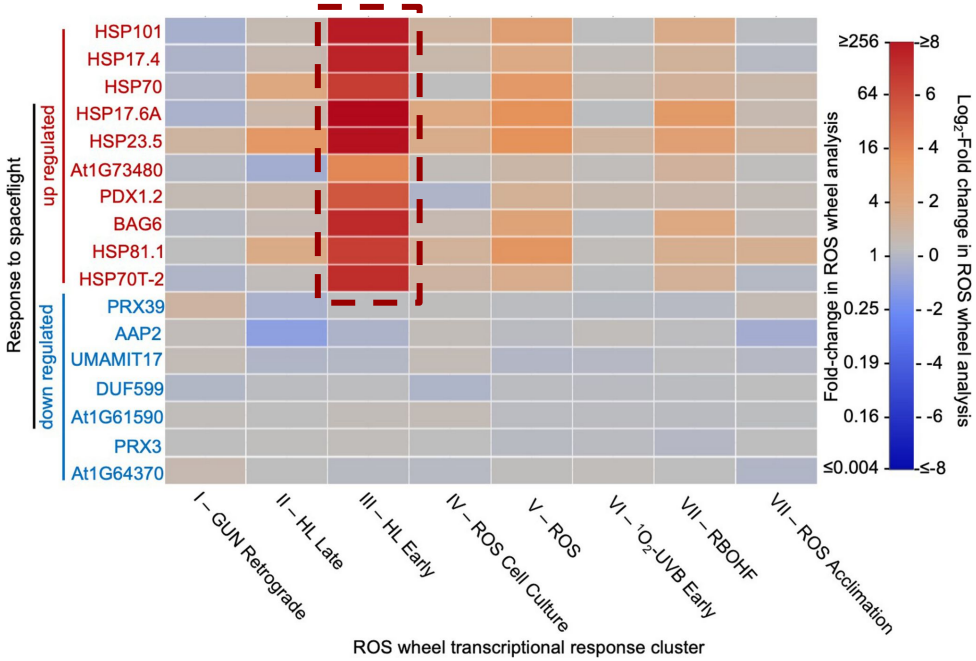


Choi et al, (2019)

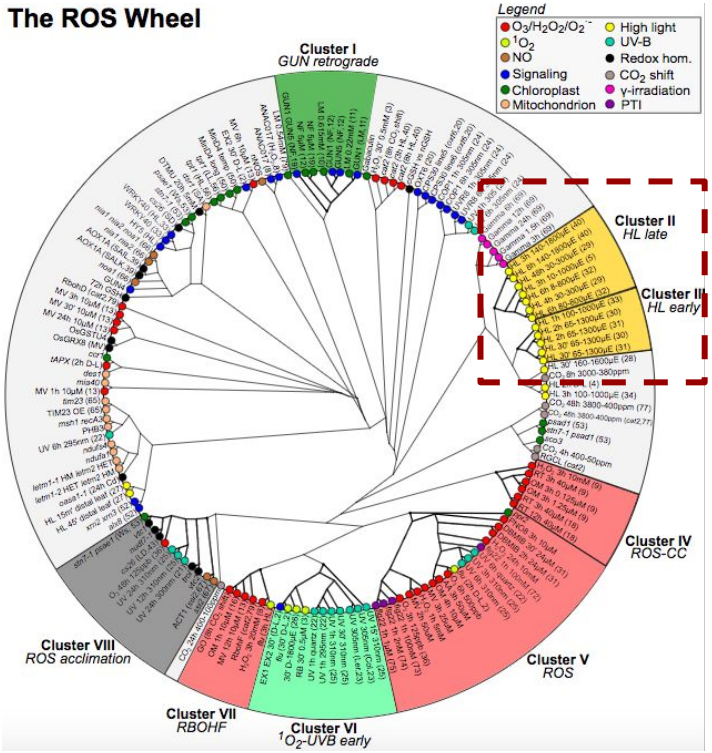


Willems et al, (2016)

Highlight response involves heatshock proteins



Choi et al, (2019)

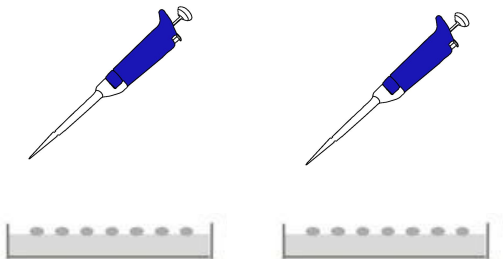


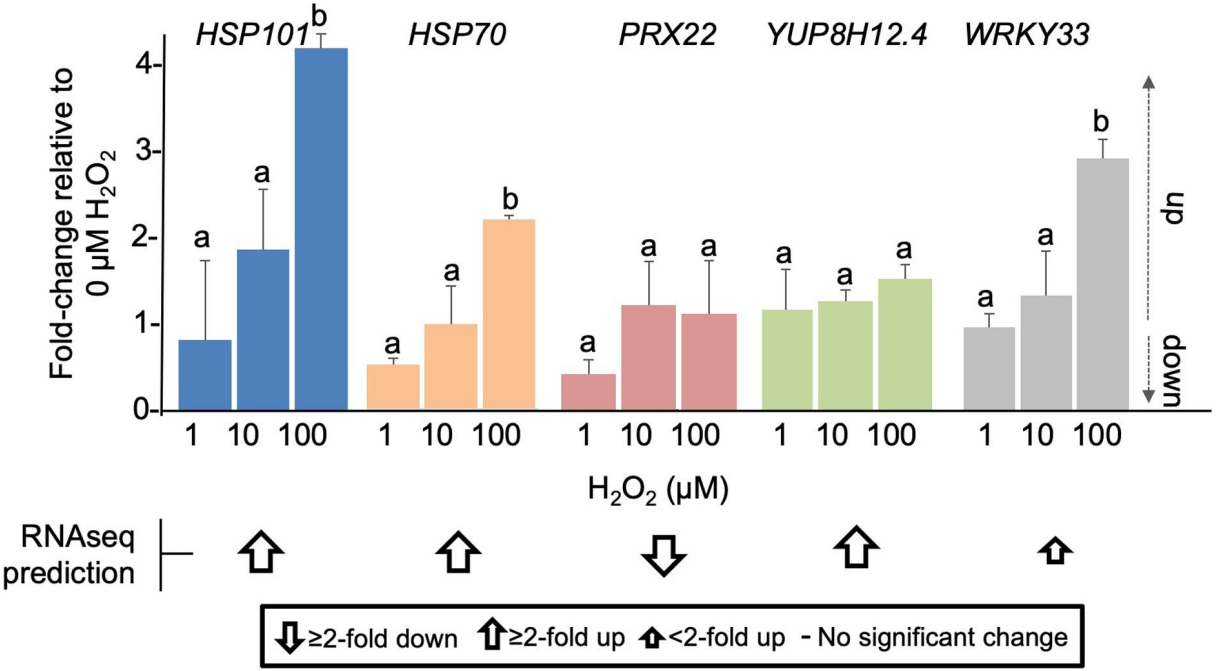
Willems et al, (2016)

ROS linked to HSP101, HSP70 and WRKY33

Water

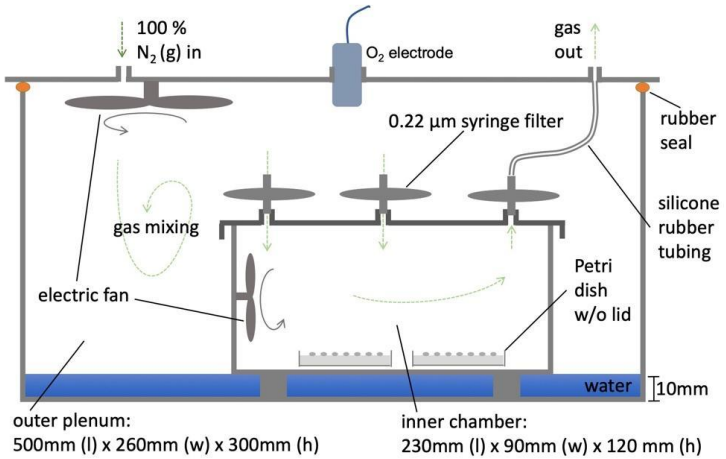
"ROS H2O2"

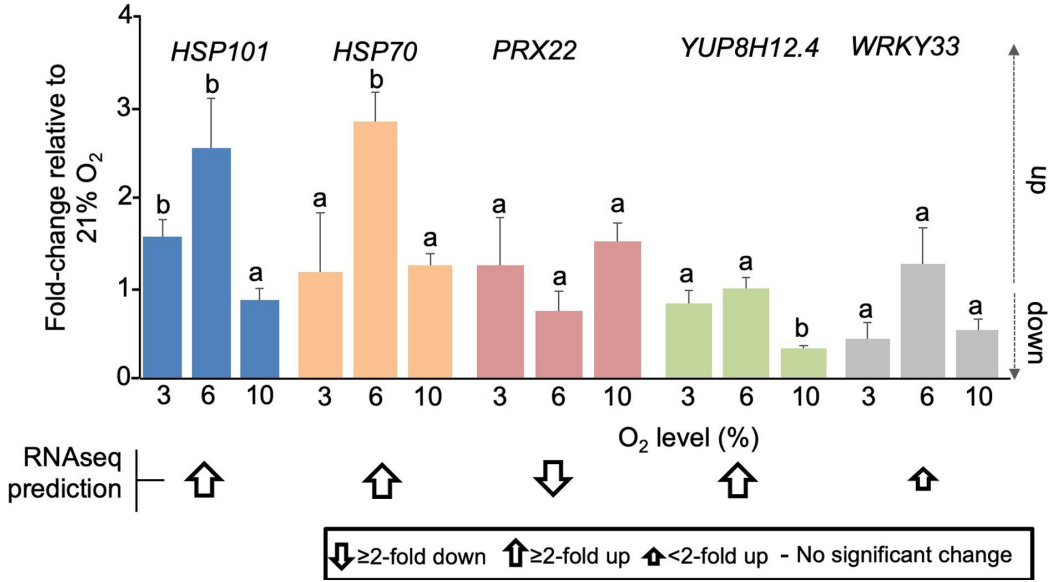




Choi et al, (2019)

Hypoxic linked to HSP101, HSP70 and WRKY33

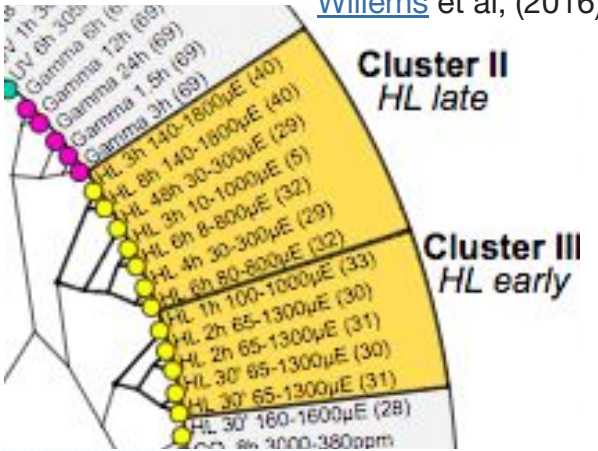


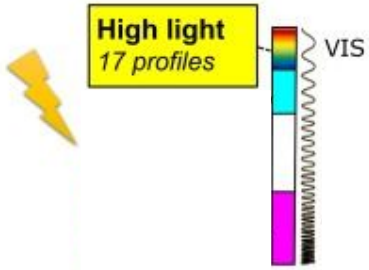


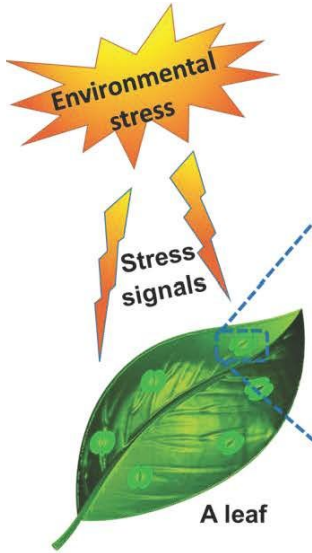
Choi et al, (2019)

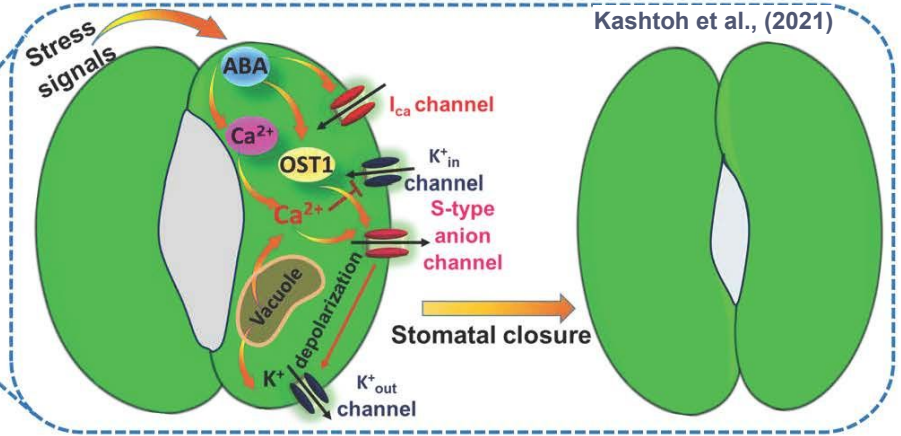
“Plants grown in darkness experience a stress similar to highlight during spaceflight”

Willems et al, (2016)





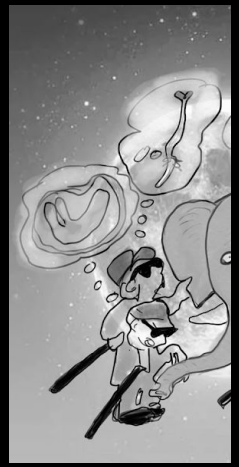




HSP’s from identified as core spaceflight response genes are involved in the response to “highlight stress”, response to ROS and hypoxia.

If *'chatGPT'* said to you "Plants grown in darkness during spaceflight experience a stress similar to highlight" wouldn't that sound odd to you?

But what about the other spaceflight studies?
Is this a truly conserved response to space flight?
Is this BRIC specific?
Is this dark specific?



Can we pull all the omics data into one relational database?

T.O.A.S.T.

We had so many questions?