WISCONSIN
UNIVERSITY OF WISCONSIN–MADISON

BRIC-17 - Mapping spaceflight-induced hypoxic signaling and response in plants

Current Project team:

- Simon Gilroy (PI, presenter)
- Dr. Won-Gyu Choi
- Dr. Sarah Swanson

Background

- Plants grow relatively 'normally' in microgravity
- Plants do show changes that suggest:
  - » Altered growth and development that is normally entrained to gravity
  - » Induction of stress responses
- Lack of buoyancy-driven convection may lead to reduced oxygen levels in unstirred regions such as the root zone

Goals

- Define global changes in gene expression patterns in Arabidopsis plants grown in microgravity
  - » using whole genome microarrays
- Compare to mutants resistant to low oxygen challenge
  - » using whole genome microarrays
  - » Also measuring root and shoot size

Outcomes

- Provide fundamental information on plant responses to the stresses inherent in spaceflight
- Potential for informing on genetic strategies to engineer plants for optimal growth in space

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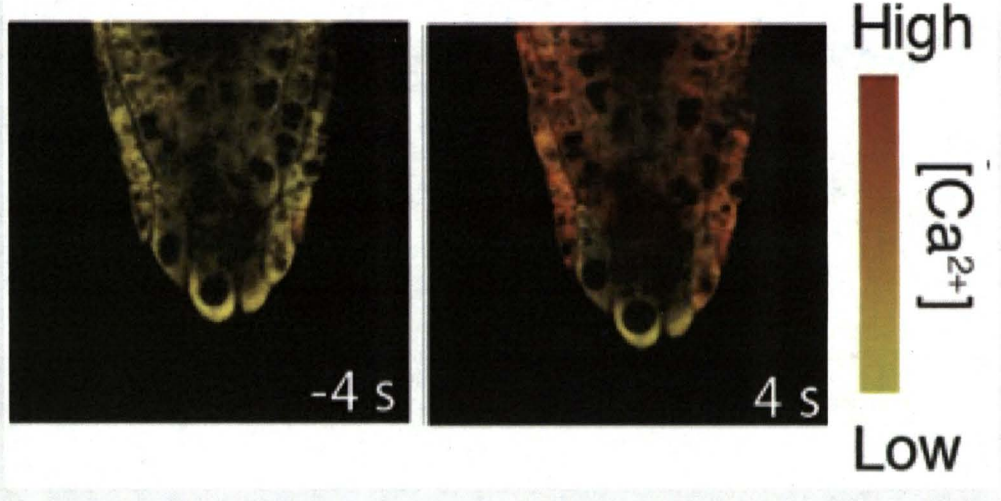
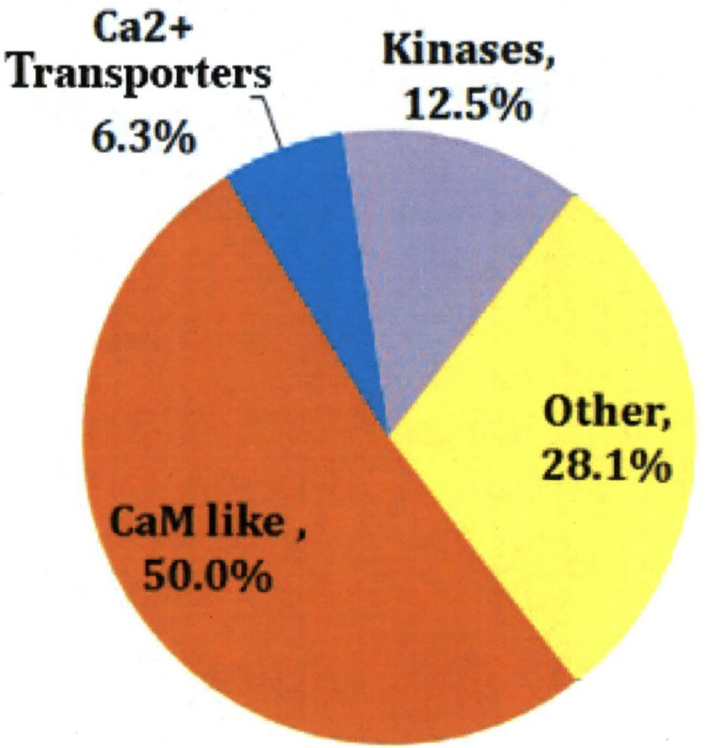
Many stress responses, including low oxygen stress, are signaled by changes in calcium ions in plant cells

Root tip Ca^{2+} changes upon low oxygen stress



Selecting candidate genes for study

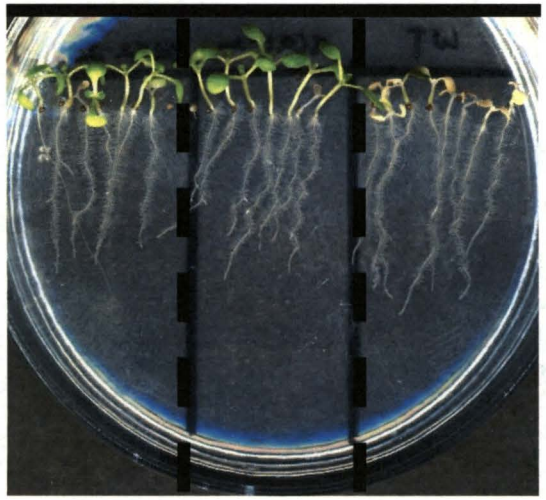
- Mined publicly available databases for genes showing altered expression in response to reduced oxygen on Earth
- Filtered for genes:
  - expressed in roots
  - related to Ca^{2+} signaling
- Independently confirmed changes in expression of candidates by qPCR analysis



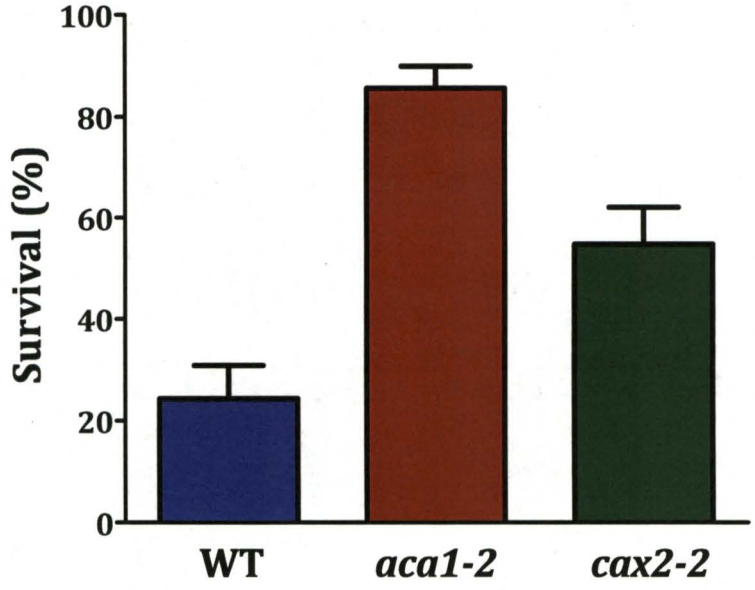
32 genes

Mutants in the Ca^{2+} transporters ACA1 and CAX2 are resistant to low oxygen stress

4 days after
0% O_2 treatment



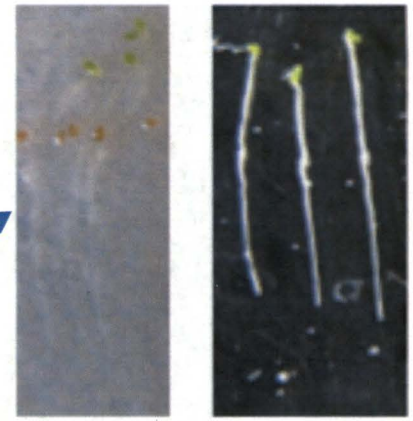
cax2-2 | ***aca1-2*** | **WT**



Experimental profile

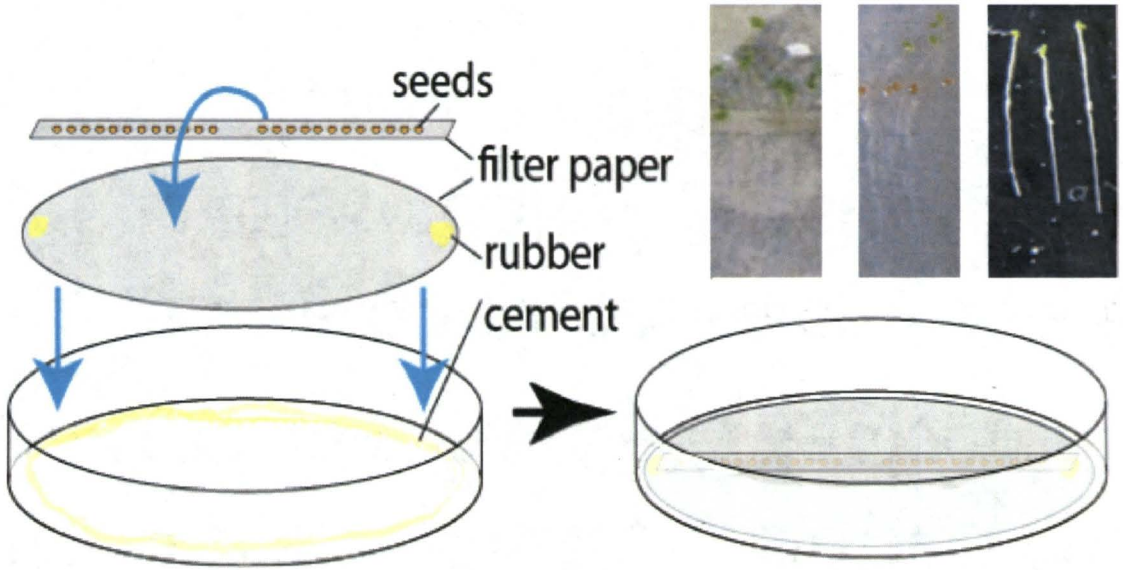
1) Load seeds (wild-type and mutants) into PDFUs dry and ungerminated
2) Transport to orbit in BRIC in ungerminated state
3) Germinate on the ISS by injecting water
4) Grow for ~8 days in dark under ambient station conditions
5) Fix *in situ* by injecting RNAlater preservative
6) Freeze and store
7) Sample return (cold)
8) Ship frozen to UW Madison for analysis

Analysis



1) Image subset of seedlings
2) Dissect into root and shoot samples
3) Isolate preserved RNA/perform DNA microarray analysis (UW Madison)
4) Compare to ground-based controls at fixed oxygen levels to define possible level of low oxygen challenge on orbit

Wicking System for germination on orbit



- Allows experiment to be supplied dry as ungerminated seeds
- Stable for several weeks
- Facilitates dissecting root from shoot
- Preserves position of each seedling allowing different mutants and wild-type to be placed on same plate – improves robustness of analysis

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