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Optimizing Growth Conditions for the Study of Plant Gravity Perception on the ISS

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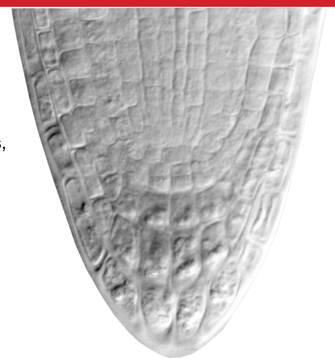
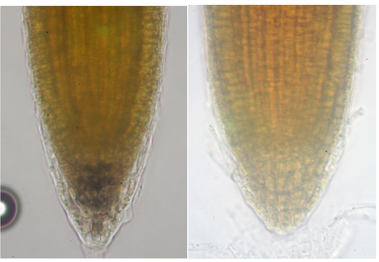
Science Background

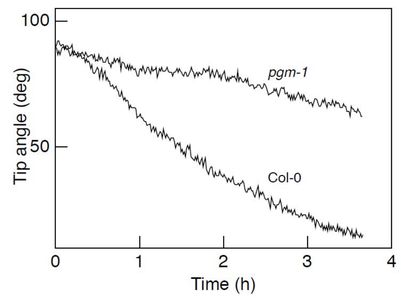
Most evidence suggests plant roots sense gravity in specialized, highly polarized cells of the central columella in the root cap, shown at right. These cells contain amyloplasts, specialized plastids filled with starch that act as statoliths, sedimenting to the lowest point of the columella cells. As roots grow and are displaced from vertical, amyloplasts sediment and initiate differential growth, returning the primary root to a vertical orientation.

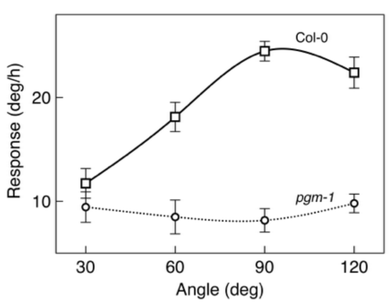




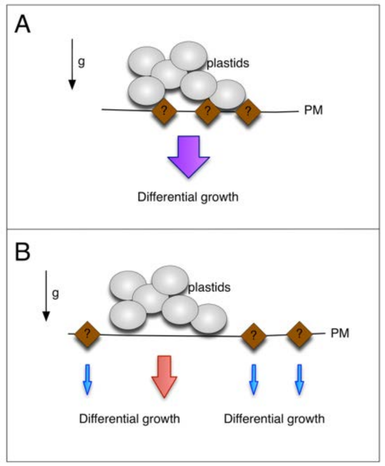
In the model plant *Arabidopsis thaliana*, a mutant has been identified with a defective gene encoding an enzyme involved early in the starch synthesis pathway. Roots with normal starch biosynthesis show dense staining with I_2KI in the columella (far left), while those of the mutant show clear root caps with no dense staining (near left). This mutant provides a way to test the contribution of plastid sedimentation to gravity sensing. We predicted that roots that lack a full complement of starch will lack gravity perception.

Previous work in our lab and others has shown that starchless roots, while significantly impaired in gravity response, nonetheless retain the ability to respond to gravity, as shown by the comparative time-course of response at right (reproduced from Wolverton et al. 2011). When starchless root tips are constrained at a stimulated angle relative to the gravity vector, they show a rate of gravitropic response that is independent of the angle of stimulation. This contrasts sharply with the behavior of wild type roots, which show a strong dependence of response rate on stimulus angle, as shown at right (reproduced from Wolverton et al. 2011). These and other observations suggest that starchless roots retain a robust, functional gravity sensing system independent of statolith sedimentation. ***Our flight experiment seeks to characterize the acceleration threshold and overall contribution of this system to the regulation of differential growth in response to gravity stimulation.***

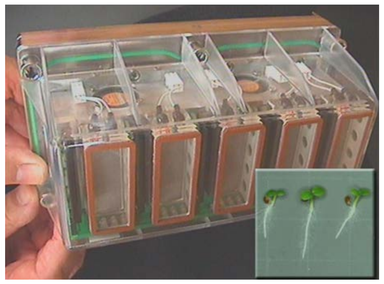




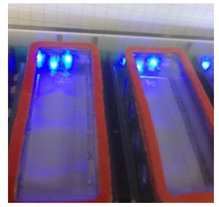
In one model of interaction between multiple gravity sensory systems, the sedimentation of plastids works in conjunction with a non-statolith system to potentiate a ***single output signal*** that controls differential growth (A). An alternate model is that the multiple gravity sensory systems act independently to modulate ***multiple output signals*** that control differential growth independently (B). The use of the microgravity environment on the ISS, coupled with the observation of wild type and starchless root responses to fractional *g* in Seed Cassettes inside the European Modular Cultivation System will allow us to test these two models of gravity perception.

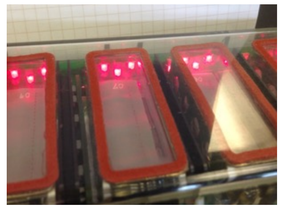


Technical Overview

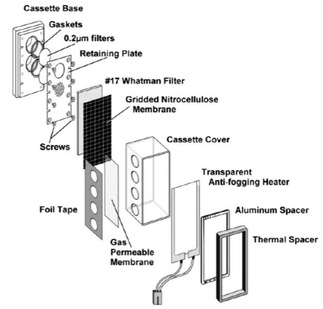


The EMCS is installed in EXPRESS rack 3A in the Destiny module of the ISS. Experiment containers (top right) can hold 5 Seed Cassettes for the growth of Arabidopsis seedlings. These growth chambers provide unilateral blue (below left) or red (below right) illumination.





EMCS Seed Cassettes are a unique growth environment for *Arabidopsis*. Seeds are affixed to a membrane substrate mounted atop filter paper, enclosed with an optically clear, transparent cover, and launched dry. Water is delivered on command into the cassette after the Experiment Container is installed in the EMCS, initiating the experiment.



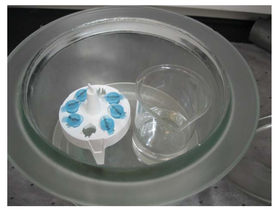
After an experiment is initiated by hydration, growth is supported by integrated white LEDs on the long axis of the Seed Cassette. The orientation of these lights provides light parallel with the artificial gravity vector supplied by the EMCS centrifuge rotor. In our experiment, seedlings will germinate and grow for a period on flight at nominal 1 *g* before experiencing microgravity and unilateral blue illumination. This treatment will induce root curvature away from the light source, resulting in roots in a stimulated orientation for the application of fractional gravity.

Flight Definition Experiments

1. After confirming the *pgm-1* mutant phenotype by staining for starch with I_2KI, we began a program of seed bulking in order to maintain seed stocks aged ≤ 6 months of age.

In addition to the phenotype screen for the absence of starch, we have also developed allele-specific PCR primers to distinguish the *pgm-1* allele from the wild type at the *PGM* locus. This not only allows us to confirm the homozygous *pgm-1* genotype, but also allows us to detect any unintentional outcrossing of the *pgm-1* allele resulting in the formation of heterozygotes.

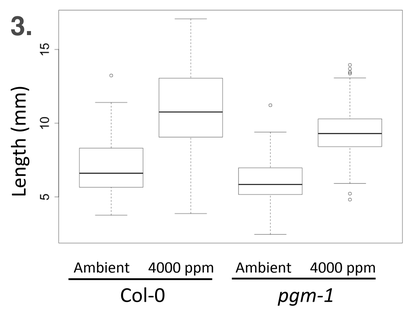
2. We confirmed a method of seed surface sterilization that exposes seed for 3 h to Cl_2 gas generated from reacting household bleach (NaOCl) with HCl under controlled conditions in a bell jar. This 'dry' sterilization removes potential contaminants without exposing the seed to an aqueous solution, which should increase germination rate.



Objectives

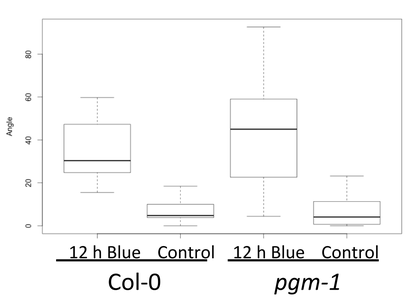
1. Confirm *pgm-1* mutant allele and seed quality
2. Test seed surface sterilization methods to optimize germination
3. Investigate the effect of elevated CO_2 on seedling growth
4. Confirm robust root phototropism on membrane substrate
5. Identify the optimal growth media composition
6. Test seed viability after long-term storage on membrane

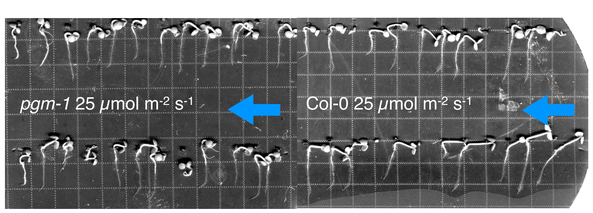
3.



On the ISS the concentration of CO_2 in the atmosphere ranges between 2000 and 4000 ppm. Since one of the principle subjects of our experiment makes use of a mutant in the carbon assimilation pathway, we sought to determine whether elevated CO_2 would show an interaction effect with the *pgm-1* lesion. We measured total root length after 4 d at 4000 ppm CO_2 and compared it to that at ambient CO_2. We found a significant increase in root length due to elevated CO_2 but no evidence of an interaction between high CO_2 and the *pgm-1* allele. Boxplot represents the median (central line), 1st and 3rd quartile (box), range (whiskers) and outliers (>1.5X IQR); n ≥ 80 for each box.

4. Although numerous studies have demonstrated phototropism in *Arabidopsis* roots, we sought to confirm this response in the unique growth conditions of the Seed Cassette. Our tests demonstrated a strong root phototropism response, with median tip angles after 12 h between 30 deg (Col-0) and 43 deg (*pgm-1*), both of which were significantly displaced from vertical ($F_{1,1}$ = 86.2, p < 0.0005). See (3) for explanation of boxplot symbols.





For our flight experiment, we plan to use directional blue light treatment to re-orient roots in the artificial *g* field in order to study the kinetics of response to fractional *g* applications. In our lab, roots of both wild type and *pgm-1* showed a strong growth response away from a directional blue (450 nm) light stimulus (25 μmol m^{-2} s^{-1}) after 12 h of stimulation. Arrows indicate the direction of light application.

Conclusions & Next Steps

We have completed 5 of the 6 objectives identified for our Flight Definition phase. We continue to work toward completing the outstanding objective, which is to test storage longevity of our seed stocks mounted and dried on the membrane-based growth system in conditions that mimic stowage during handover, launch, and stowage on the ISS.

Acknowledgements

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