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

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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 I2/KI 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.

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Science Background

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 (B).

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.

Flight Definition Experiments

1. After confirming the pgm-1 mutant phenotype by staining for starch with I2/KI, we began a program of seed building 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 Cl2 gas generated from reacting household bleach (NaOCl) with HCl under controlled conditions in a bell jar. This Cl2 sterilization removes potential contaminants without exposing the seed to a toxic solution, which should increase germination rate.

3. A comparison of the wild type with the pgm-1 allele showed a strong growth response away from a directional light stimulus (25 µmol m-2 s-1). This strong response indicates that the pgm-1 allele is unique to Arabidopsis. These results are shown in the Boxplot below.

4. Although numerous studies have demonstrated photosynthetic properties in Arabidopsis roots, we sought to confirm this response in the unique growth conditions of the Seed Cassette. Our tests demonstrated a strong root photosynthetic 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 (P<0.0005). See (3) for explanation of boxplot symbols.

Technical Overview

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 at 15 g before experiencing microgravity and unilateral blue illumination. This treatment will induce root curvatures away from the light source, resulting in roots in a stimulated orientation for the application of fractional gravity.

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 storage during handover, launch, and storage on the ISS.

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