# Space Life Sciences Research Highlights

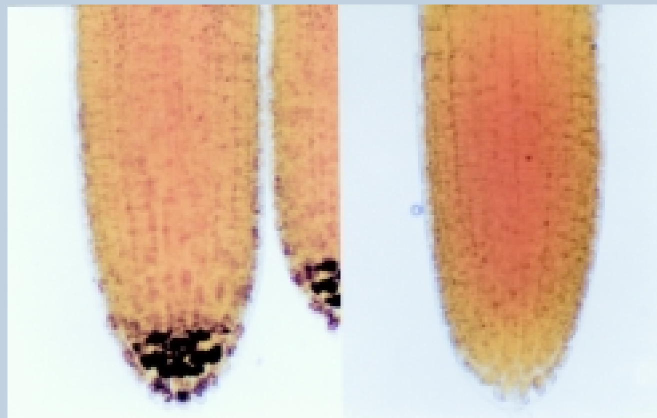
## Shuttle Experiments Are Improving Our Understanding of How Plants Perceive Gravity

*The results of two experiments carried out on the space shuttle are providing important clues not only about how plants detect gravity on Earth but also about how plants might one day be grown in space. The experiments were designed and directed by NASA-supported investigator John Kiss.*

Why do plant roots grow downward and plant stems grow upward? Simply put, it is because plants can sense gravity. However, the precise cellular mechanisms by which plants perceive and respond to gravity are controversial among scientists.

"There are two competing hypotheses about how plants perceive gravity," explains John Kiss, Professor of Botany at Miami University in Oxford, Ohio, a NASA-supported investigator whose laboratory focuses on the cellular mechanisms that form the basis of gravity perception in plants.

The "starch-statolith" hypothesis, which was first proposed about 100 years ago, holds that dense starch bodies known as amyloplasts, found in cells in the root cap, are the plant gravity sensor. Experiments have shown that when a root is turned on its side, within hours it will begin to bend downward. The starch bodies quickly tumble to the new bottom of the root, appearing to point the way down.



*Roots of normal (wild-type) Arabidopsis seedlings (left) stain positively for starch with iodine, while there is no reaction with the root of a starchless mutant (right). Seedlings of these strains developed in space during shuttle missions STS-81 and STS-84.*

In the 1980s, scientists tested the starch-statolith hypothesis by creating genetically altered plants with fewer starch bodies in the root cap. They found that the roots of these mutant plants grew downward anyway, even with less starch. These observations along with other experiments gave rise to a new hypothesis about how plants perceive gravity—the "protoplast pressure" hypothesis. According to this model, the weight of the entire contents of the cells in the root cap acts as the gravity sensor.

NASA is interested in understanding how plants perceive and respond to gravity because plants will play a significant role in any future long-term space missions or efforts to colonize the moon or other planets. "Plants are important for two reasons," says Kiss. "They are a food source and they generate oxygen."

Because of the constant presence of gravity on Earth, the mechanisms of gravity perception cannot be determined solely through Earth-based experiments. "To really study how plants perceive gravity, you have to take gravity away and give it back in measured quantities," says Kiss. That is exactly what he and his colleagues did in two experiments carried out on the space shuttle in 1997. Their goal was to test the starch-statolith hypothesis in the unique environment of microgravity.

The experiments used a facility called Biorack that was developed by the European Space Agency to study single cells, plant seedlings, and small invertebrate animals in space. Kiss's group conducted a small, preliminary experiment in January 1997 during shuttle mission STS-81. Their main experiment, involving a much larger number of plant seedlings, was conducted five months later in May 1997 on STS-84.

Both experiments employed four variants of *Arabidopsis thaliana*, a type of mustard plant that is widely used in plant genetics research. One was a "wild type" plant that contained the normal complement of starch bodies. Three variants had genetic alterations: two contained reduced numbers of starch bodies and the third none at all.

"We sent up dry seeds," explains Kiss. "The astronauts watered them and they developed into seedlings." The astronauts then exposed the seedlings to measured quantities of gravity in a centrifuge, a machine that produces gravitational effects. Meanwhile, Kiss and his associates conducted an identical control experiment in a Biorack unit on the ground at the Kennedy Space Center. After the shuttle returned to Earth, the investigators carefully analyzed the responses of the stems to gravity for both sets of seedlings.

"We observed an increased response to artificial gravity in the seedlings containing more starch, says Kiss." The wild-type (normal) seedlings showed the strongest response; the weakest response occurred in the starchless mutants.

However, there was a problem: ethylene gas, which at low levels is harmless to humans but causes diminished growth in plants, was present in the spacecraft, from an unknown source. Kiss and his colleagues had to perform additional control experiments on the ground to exclude the possibility that the observed differences in the seedlings' response to artificial gravity were due to the presence of ethylene.

"We showed that ethylene slowed down the rate of growth equally in all the seedling variants," says Kiss. Thus, the results of the shuttle experiments strengthen the hypothesis that starch bodies are the plant gravity sensor.

Seedlings grown on the shuttle also exhibited decreased starch content, compared with plants grown on Earth. Previous experiments by other groups had suggested that this reduction might be caused by microgravity. However, the flight centrifuged seedlings mimicking Earth's gravity showed reduced starch, just like the flight microgravity seedlings, suggesting that microgravity was not the cause of the reduced starch. Kiss and his colleagues also found that control plants grown in an environment with added ethylene had reduced starch content.

"Our data show that any alterations in starch are due to atmospheric problems with ethylene, not to microgravity per se," says Kiss. These problems demonstrate that, if plants are to be successfully grown on spacecraft in the future, the onboard atmosphere must be carefully controlled to avoid ethylene contamination.

Kiss and his associates are also trying to characterize the molecular mechanisms by which plants respond to gravity after perceiving it. They are focusing on the role of plant proteins that resemble integrins, proteins that in animals are located in the cell membrane and are involved in intracellular signaling. In plants the function of these integrin-like proteins is not well understood.

*John Kiss standing between astronauts John Grunsfeld (left) and Jeff Wisoff (right) during crew training at KSC. These two mission specialists performed the Arabidopsis experiments during STS-81.*

Kiss and Lucinda Swatzell (who is supported by a NASA Graduate Student Researchers Program fellowship) recently showed that in *Arabidopsis*, integrin-like proteins are located in the cell membrane. "This suggests that intracellular signaling mechanisms may operate in a similar manner in plant and animal cells, but further research is needed to establish this," says Kiss. "Although plant and animal cells seem to be very different, more and more similarities are being found in their basic biochemical composition. It's very exciting."

Kiss notes that in the future, understanding how plants perceive and respond to gravity could have practical applications. "All plants have to orient themselves up to get light and down for anchorage. If we understood how this process works, we might be able to do genetic engineering to improve crop productivity."

References


1. Kiss JZ; Edelmann RE; Wood PC. Gravitropism of hypocotyls of wild-type and starch-deficient Arabidopsis seedlings in spaceflight studies. *Planta* 209(1):96-103, 1999.
2. Guisinger MM; Kiss JZ. The influence of microgravity and spaceflight on columella cell ultrastructure in starch-deficient mutants of Arabidopsis. *Am J Bot* 86(10):1357-66, 1999.
3. Kiss JZ; Katembe WJ; Edelmann RE. Gravitropism and development of wild-type and starch-deficient mutants of Arabidopsis during spaceflight. *Physiol Plant.* 102(4):493-502, 1998.
4. Katembe WJ; Edelmann RE; Brinckmann E; Kiss JZ. The development of spaceflight experiments with Arabidopsis as a model system in gravitropism studies. *J Plant Res.* 111(1103):463-70, 1998.
5. Swatzell LJ; Edelmann RE; Makaroff CA; Kiss JZ. Integrin-like proteins are localized to plasma membrane fractions, not plastids, in Arabidopsis. *Plant Cell Physiol.* 40(2):173-83, 1999.
6. MacCleery SA; Kiss JZ. Plastid sedimentation kinetics in roots of wild-type and starch-deficient mutants of Arabidopsis. *Plant Physiol.* 120(1):183-92, 1999.