Transgenic Plants Help Answer Questions about the Effects of Space Flight on Plant Biology

NASA-supported researchers are developing transgenic plants that can act as biological sensors of the conditions perceived by plants in microgravity. Results from the first application of this technology in space provide new evidence regarding plant stress that calls into question the hypothesis that plants suffer from oxygen deprivation during space flight.

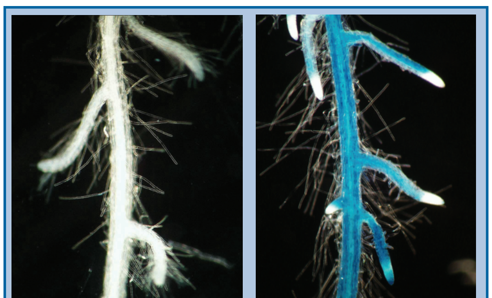
NASA-supported investigator Robert Ferl is developing plants that can tell us how they're feeling. Ferl's plants are genetically engineered to contain genes that cause cells and tissues to turn a bright color when the plants are under certain kinds of stress.

Like all living organisms, plants are continually making physiological adjustments in response to changes in their environment, says Ferl, professor of horticultural sciences at the University of Florida in Gainesville.

"Environmental changes almost always lead to changes in gene regulation," explains Ferl. "We use genetic engineering to design plants in which the adaptation response to a specific kind of stress is visible to the investigator. Our goal is to use this technology to try to answer important questions about the effects of space flight on plant biology."

Ferl, co-investigator Anna-Lisa Paul, and their colleagues have genetically altered *Arabidopsis* plants by attaching a so-called "reporter gene." A reporter gene is one that's been engineered to give a signal (such as turning a bright color) when the gene to which it's attached is activated. The reporter gene is composed of the sensory part of one gene (the Adh gene promoter) and the coding region of another that serves to generate the reporting color (known as GUS). This transgene is referred to as Adh/GUS.

Astronaut Cady Coleman examines Arabidopsis plants in one of the chambers of the Plant Growth Facility during the STS-93 mission. (NASA photo S93E5113.)



(Left) Typical Arabidopsis root. (Right) Arabidopsis root demonstrating reporter gene expression in a plant suffering from hypoxia; the blue color indicates the specific areas of the plant that have been affected by a lack of oxygen.

The sensory gene Adh is activated by stresses, including lack of oxygen, certain kinds of drought, and certain plant hormones. When the plant is experiencing any of these stresses, expression of the Adh/GUS transgene is indicated by bright blue patches that appear in the tissues under stress, such as the roots, stems, or shoots.

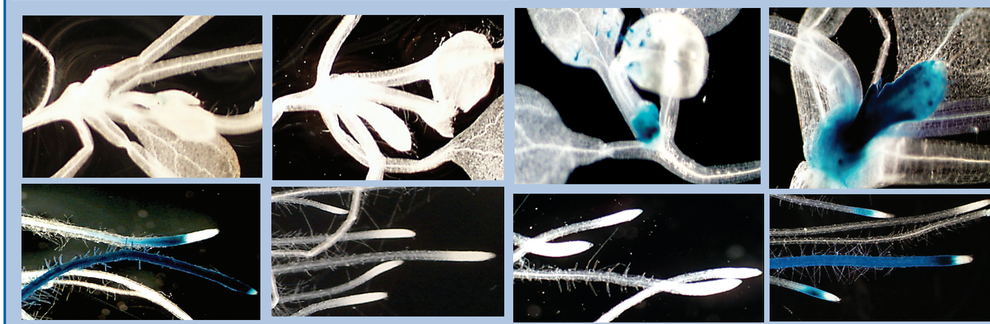
Does Space Flight Induce Hypoxia?

Space biologists have observed that plants flown in space show the same signs of stress as plants on the ground that have been deprived of oxygen. Does space flight induce hypoxia (insufficient oxygen) in plants? It seems a reasonable hypothesis, since in the absence of gravity there is no convection, or movement of gases in the atmosphere, which might lead to a lack of available oxygen. But it was also possible that what looked like hypoxia in the flight plants was in reality a response to a secondary effect of space flight.

To try to answer this question, *Arabidopsis* plants bearing the Adh/GUS transgene—known as TAGES, for Transgenic Arabidopsis Gene Expression System—were flown for five days on the STS-93 shuttle mission in 1999. Both in flight and in a control experiment on the ground, the plants were cultivated in a "stress-free" growing system to avoid background expression of the Adh/GUS transgene. This was to ensure that any signs of stress observed in the flight plants were clearly attributable to space flight. "When the plants are not stressed in any way in their growth environment, they are wonderful sensors for any stress induced by space flight," says Ferl.

The flight plants were harvested about three hours after the Shuttle landed. Both flight and control plants were chemically stained to reveal expression of the Adh/GUS transgene. The control plants showed virtually no evidence of expression of the transgene. The flight plants, by contrast, exhibited patches of blue tissue in the roots.

STS-93 TAGES (Transgenic Arabidopsis Gene Expression System) Experiment | ***Ground Experiments with Controlled Flooding***



Flight shoot apex (above) and root tips (below). Adh/GUS transgene expression in roots only.

Ground control shoot apex (above) and root tips (below). No Adh/GUS transgene expression in shoots or roots.

Mild hypoxia (10% oxygen) shows hypoxic induction of the transgene in the shoot only (above).

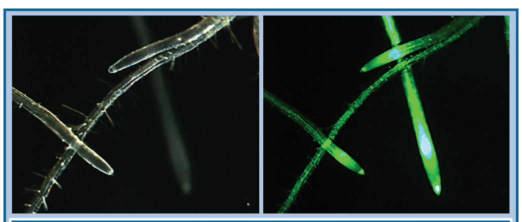
Flooded control shows hypoxic induction of the transgene in the shoot and in the root.

Ground Control Experiments Provide a Clue

To try to recreate the pattern of Adh/GUS expression seen in the flight plants, Ferl and his associates conducted an additional set of ground control experiments. Plants bearing the Adh/GUS transgene were flooded with water or mixed gasses to simulate hypoxia. Plants subjected to mild hypoxia—just cutting the amount of oxygen in half—exhibited patches of blue tissue at the apical (tip) portion of the shoot, but not in the roots. Those subjected to more severe hypoxia—flooding the entire roots with water—exhibited patches of blue tissue in the root tips as well as the apical portion of the shoot where the new leaves emerge.

"These ground experiments suggest that the shoot tip is the part of the plant most sensitive to lack of oxygen," says Ferl. "In the flight plants, we saw no evidence of Adh/GUS expression in the shoot tips. We therefore conclude that plants growing in space do not exhibit the characteristics of plants deprived of oxygen. The blue tissue seen in the roots of the flight plants suggest that something other than lack of atmospheric oxygen may be causing Adh/GUS expression, or that signals transmitted from root to shoot do not occur."

These findings offer further evidence that there is no fundamental barrier to growing plants in microgravity, adds Ferl. "It appears that plants *can* adapt to space," he says. "The challenge lies in designing an environment in which plants can thrive in microgravity. It's complicated to grow plants in space—you have to get the engineering right—but studies like these can help define the most favorable environment."



Arabidopsis roots containing the Adh/GFP transgene. Photo on left was taken in white light; photo on right shows the GFP fluorescence in 488 nm blue light.

Advantage of "Colorful" Transgenes

The use of transgenes that produce a color has a great advantage over other methods of analyzing plants genetically, says Ferl. "We can just look at the plant—or at a photograph of the plant—and see exactly where adaptive stress is occurring."

Ferl and his colleagues have developed another *Arabidopsis* plant containing a gene (Adh/GFP transgene) that will cause tissues to glow bright green in response to stresses such as hypoxia. They hope to test this plant experimentally on a future shuttle mission, as well as on the International Space Station.

On the Space Station, the investigators plan to use Web cameras to record gene expression throughout the plants' entire growth cycle in real time. "We hope to be able to observe what happens over time as the plants adapt to the space flight environment," explains Ferl. We will be able to watch the plants through their entire life cycle—seeing which genes are active and in what part of the plant they are active—in real time over the Internet."

References
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