Acronym: TAGES

Title: Transgenic Arabidopsis Gene Expression System

Principal Investigator(s):
Robert Ferl, Ph.D., University of Florida, Gainesville, FL
Anna-Lisa Paul, Ph.D., University of Florida, Gainesville, FL

Contact(s):
PI - Robert Ferl, 352-392-1928 x301
PI - Anna-Lisa Paul, 352-392-1928 x-331
Secondary - David R. Cox 321-867-6051

Mailing Address(es):
Robert Ferl, Ph.D.
Department of Horticultural Sciences
University of Florida
Gainesville, FL 32601

Anna-Lisa Paul, Ph.D.
Department of Horticultural Sciences
University of Florida
Gainesville, FL 32601

Developer(s): Kennedy Space Center, FL
The Bionetics Corporation, Kennedy Space Center, FL

Sponsoring Agency: National Aeronautics and Space Administration (NASA)

Increment(s) Assigned: 19, 20

Brief Research Summary (PAO): The Transgenic Arabidopsis Gene Expression System (TAGES) investigation is one in a pair of investigations that use the Advanced Biological Research System (ABRS) facility. TAGES uses Arabidopsis thaliana, thale cress, with sensor promoter-reporter gene constructs that render the plants as biomonitors (an organism used to determine the quality of the surrounding environment) of their environment using real-time nondestructive Green Fluorescent Protein (GFP) imagery and traditional postflight analyses.

Research Summary:

- Previous on-orbit plant experiments that examined stresses at the genetic level involved terminating plant growth for analysis. Transgenic Arabidopsis Gene Expression System (TAGES) uses a new real-time imaging system in conjunction with a genetically modified plant that removes the need for harvesting thereby allowing the plant to continue to grow and making it possible to follow the development of stress in a plant over time.

- TAGES uses Arabidopsis thaliana, commonly known as thale cress, to determine how plants perceive stresses in the space flight environment such as drought, inadequate light, or varying temperatures.

- The new real-time technique uses a gene inserted into the genome of the plant. When a plant perceives a certain stress, it expresses this gene. The gene expression can be viewed with a special camera once the plant is illuminated with a certain frequency of blue light, the plant fluoresces green.
• Such genetically modified plants and imaging tools could be used as 'biosensors' for characterizing other spacecraft environments. These same tools could also be used to further develop and analyze plants that could grow in either lunar or Martian bases.

**Detailed Research Description:** The Transgenic *Arabidopsis* Gene Expression System (TAGES) investigation will provide an understanding of physiological processes such as gene expression, metabolism and general plant development that are affected in plant systems exposed to space flight.

TAGES investigation seeks to understand space-flight induced molecular changes in *Arabidopsis thaliana* gene activity. A series of transgenic plants (plants containing foreign DNA integrated into their genome) have been designed for the TAGES investigation. The plants carry sensor promoter-reporter gene constructs that are capable of monitoring a variety of environmental and developmental influences, thereby rendering the plants biomonitor of their environment. *Arabidopsis thaliana* is the plant of choice to house the sensor promoter-reporter gene constructs due to its well-understood genome and relatively short seed-to-seed cycle, as well as having been the focus of several space-flight studies on previous plant experiments conducted during ISS, Mir and Shuttle missions.

The first group of biomonitors for TAGES consists of plants with alcohol dehydrogenase (Adh) sensor promoter and beta-glucuronidase (GUS) reporter gene constructions. The second group of biomonitor plants incorporates the Green Fluorescent Protein (GFP) reporter gene construction. Two primary goals have been identified for the TAGES experiment: 1) confirm and extend data from an experiment conducted on STS-93 in 1999 by utilizing the GUS reporter gene system, 2) test the fidelity and practicality of the GFP reporter gene system in comparison to GUS.

The GFP Imaging System (GIS) will demonstrate a powerful real-time, non-destructive analytical tool that can be used to assess the status of a target organism. This device will help to revolutionize space-based biological research by ultimately eliminating the resource-intensive need to return biological material to Earth for postflight analysis. This advanced technique can be applied to a host of model organisms engineered with the GFP gene construct including plants, microbes, and nematodes.

**Project Type:** Payload

**Images and Captions:**

*Advanced Biological Research System (ABRS) Green Fluorescent Protein (GFP) Imaging System prototype. Image provided by The Bionetics Corporation at Kennedy Space Center.***

*Arabidopsis* plants imaged in white light (left) and Green Fluorescent Protein (GFP) excitation illumination, right. Image provided by Anna-Lisa Paul, Ph.D. and Robert Ferl, Ph.D., Department of Horticultural Sciences, University of Florida.

**Operations Location:** ISS Inflight
Brief Research Operations:

- The TAGES, *Arabidopsis thaliana* plants are launched live along with the powered Advanced Biological Research System (ABRS). The first TAGES beta-glucuronidase (GUS) harvest occurs at Launch plus 5 (L+5) days using the Kennedy Space Center (KSC) fixation tubes (KFT) preservation (RNAlater®).

- The *A. thaliana* plants containing the Green Fluorescent Protein (GFP) reporter gene are autonomously imaged within the ABRS facility using the GFP Imaging System (GIS).

- About every 2 weeks, GFP plants are harvested and other plants within ABRS are moved into the field of view of the GFP camera.

Operational Requirements: TAGES requires a controlled environment provided by the ABRS facility which also provides images that are downlinked to the ground teams. The crew is responsible for harvesting, reinitialization, water refill and changing out the air filter. After harvesting, parts of the samples are chemically preserved and stored in the Minus Eighty-Degree Laboratory Freezer for ISS (MELFI).

Operational Protocols: The crewmembers are responsible for refilling the water reservoir by using a syringe to transfer approximately 60-mL of water from the ISS potable water source to each of two quick disconnect fittings associated with the two reservoirs inside the ABRS. Air filter change out is performed by opening the front hatch of the ABRS locker, loosening a Velcro restraining strap, and pulling each of the two filters off of the back side of the hatch. There are blind mate connectors on the back side of each filter.

For the harvesting of the TAGES *A. thaliana* plants, crewmembers remove one root tray from the ABRS to access petri plates containing the plants. Plants are harvested from petri plates into the KFTs. Some of these KFTs require a day to perfuse the plant tissues before placing in cold stowage at -68 degrees C or colder until return. During the harvests, some petri plates are moved into or out of the field of view of the autonomous GFP Imaging System (GIS) attached to the root tray.

Review Cycle Status: PI Reviewed

Category: Biological Sciences in Microgravity

Sub-Category: Plant Biology

Space Applications: TAGES along with the ABRS hardware demonstrates the capabilities of providing the correct environment for plant growth onboard spacecraft. For future long-duration exploration, crews will need to be able to grow plants for a variety of applications.

Earth Applications: The miniaturization of the Green Fluorescent Protein (GFP) imaging apparatus as a requirement for this space flight investigation has produced a device that is easily transportable and may be used as a means for conducting *in situ* analysis of appropriately genetically prepared biomonitors. An engineering prototype of this device has already been used by a joint U.S. and Canadian research team to conduct biomonitoring of plants at the remote Haughton crater in the high arctic on Devon Island, Nunavut, Canada. The TAGES system also represents advances in telemetric data collection. There are numerous biological and biomedical applications for this type of technology in terrestrial applications.

Manifest Status: Continuing

Supporting Organization: Exploration Systems Mission Directorate (ESMD)
Previous Missions: A similar investigation, Plant Growth in Microgravity (PGIM-01), flew onboard STS-93 in 1999.

Related Payload(s): Cambium

Last Update: 02/12/2009