USE OF INDUCED-FLUORESCENCE IMAGING AND GREEN FLUORESCENT PROTEINS TO MONITOR THE HEALTH OF TERRESTRIAL PLANTS UNDER SIMULATED MARTIAN ENVIRONMENTS

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INTRODUCTION

Advanced Life Support (ALS) systems using higher plants have been proposed for Human missions to Mars. The ALS systems could dramatically reduce launch costs from Earth by regenerating oxygen, water, and food. However, no information is currently available on how terrestrial plants will grow under simulated Martian environmental conditions. The proposed research will address the following three questions. Is Martian regolith capable of supporting robust plant growth? Can low-pressure environments be designed for optimizing plant growth? Can a miniaturized remote sensing system be developed to monitor plant health within low-pressure environments in which plants are stressed by soil factors likely to be found in Martian regolith? To answer these questions a series of experiments will be conducted at Earth-normal (101 kPa) and low (10-40 kPa) pressures in which genetically engineered plants of Arabidopsis thaliana will be grown in an analog Mars soil supplemented with different stressing agents. Arabidopsis thaliana plants will be genetically modified with green fluorescent proteins (GFP) linked to plant reporter genes in order to monitor specific physiological pathways in plants affected by specific soil stressing agents.

CURRENT STATUS OF RESEARCH

A) Remote Sensing Imager: A remote sensing imaging (RSI) system will be developed to collect plant stress data throughout these experiments. The RSI system will use a single charged-couple device (CCD) camera fitted with five unique narrow-bandpass filters in a rotating filter-wheel. The CCD camera will capture images at a resolution of 768 x 494 pixels. The RSI camera will be fitted with the following filters: 440 (blue), 525 (green), 685 (red), 735 (far-red), or 750 (far-red) nm (± 10 nm). Images from the five filters will provide data for a number of image-analysis approaches. First, images from the 440, 525, and 685 nm filters will be processed to yield standard RGB color images of the plants. Second, images from the 685 and 750 nm filters will be used to calculate standard spectral reflectance ratios (e.g., NDVI values, R750/R685 ratios, and logR750/logR685 ratios) in order to estimate general plant stress in visible wavelengths. [NDVI = normalized difference vegetation index.] Third, fluorescent images from the 685 and 735 nm filters will be processed to yield red:far-red ratio images for detecting general plant stress under fluorescent illumination. Fourth, green fluorescent images will be collected with the 525 nm filter under fluorescent illumination to measure green fluorescent protein (GFP) expression in transgenic plants. The RSI system will be used to capture images from non-transgenic and transgenic lines of A. thaliana grown under various stresses including low pressure and the presence of soil peroxides or heavy metals.

B) Use of Green Fluorescent Proteins (GFP) as Marker Genes: The expression of GFP is controlled by stress-induced reporter genes linked to specific GFP-constructs in transgenic lines of A. thaliana. By carefully selecting the stress promoters, information will be obtained on the function of key physiological pathways in plants that are affected by specific soil stressing agents. Furthermore, the use of these GFP-
tagged stress promoters in plants should allow the transgenic lines of *A. thaliana* to serve as bioassays for specific classes of compounds in regolith during the Mars 2005 HEDS lander mission. The stress reporter genes will be: *Cat* (catalase), *Adh* (general plant stress gene), and *Hsp/Gst* (heat-shock) which are specific for the presence of soil peroxides, oxidants or anoxia, and heavy metals, respectively.

C) Effects of Low Pressure on Plant Growth: The surface ambient pressure on Mars is 6-8 mb (approximately 0.7 kPa); a pressure that is not conducive to the growth of terrestrial plants. However, if a low-pressure environment (e.g., 20-40 kPa) were identified in which normal plant growth can be achieved; then the structural, volume, and gas-consumable requirements of a Mars lander plant growth experiment might be reduced. Several plant functions are affected by growth under low pressures including, photosynthesis, evapotranspiration, respiration and water use. But very little research has been conducted on plant growth and development in pressures below 70 kPa (70% of the ambient pressure on Earth). This research will study the limits of growth for the terrestrial plant, *Arabidopsis thaliana*, under low atmospheric pressure (10-40 kPa) and altered gas compositions. Two main questions will be addressed in this portion of the work: a) what is the minimum total ambient pressure and b) what are the proper partial pressures of CO₂ and O₂ required to maintain normal plant growth.

RESULTS

The RSI system is currently in the initial design and testing phases of operation. The primary objective for the first 6 months of this project has been to determine the sensitivity of each spectral band in both the spectral reflectance and fluorescence mode. Results indicate that the CCD chip used in the RSI system is sensitive enough in the spectral ranges used for these tests to accommodate all measurements listed above. Plant biology experiments are currently ongoing, and results will be presented at the Bioastronautics conference.

As of this writing, both the plant transgenic and low-pressure experiments were scheduled for the fall of 2000. Results of these experiments will be discussed at the Bioastronautics conference in January, 2001.

FUTURE PLANS

The research discussed above will be conducted in separate but parallel efforts. But the objective for Year-2 (June, 2001-June, 2002) activities will be to develop a series of integrated experiments conducted at low pressure within a 1 cubic meter Mars Simulation Chamber (MSC) located at the Kennedy Space Center, FL. Experiments will be conducted in the MSC system in which GFP-transgenic plants will be grown at pressures lower than terrestrial ambient conditions in Mars analog soils treated with peroxides or heavy metals. The integrated experiments will be designed to confirm the effectiveness of the GFP and fluorescence imaging system to measure plant stress induced by soil factors on plants grown at low pressures. The primary soil factors selected for this phase of the research (soil peroxides and heavy metals) are those anticipated (based on the literature) as posing significant risks to inducing plant stress during a plant biology experiment on future Mars lander missions. Transgenic lines of *A. thaliana* will be grown at the total atmospheric pressure (expected to be between 20-40 kPa) and partial pressures of O₂ and CO₂ determined by the research described above. The expression of GFP genes (i.e., as they are expressed while linked to reporter genes) are hypothesized to be capable of detecting soil stressing agents at lower concentrations than measuring whole-plant physiological changes induced by the same stressing agents and using standard methods of remote sensing (e.g., NDVI, R750/R685, logR750/logR685, or red:far-red ratios).

INDEX TERMS
Remote sensing, leaf fluorescence, spectral reflectance, green fluorescent proteins, GFP, anoxia, hypoxia, plant physiology, Arabidopsis thaliana, Mars, simulated soils, Mars regolith, low-pressure environments