Lunar Plant Experiment: Testing plant germination in lunar gravity and ionizing radiation

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Summary:

We present an open design for a first plant growth module on the Moon (LPX). The primary science goal of lunar habitat is to investigate germination and initial plant growth when subject to the combined effects of lunar gravity and lunar surface radiation. The LPX module has been designed to be a flexible base unit that can be adapted to fly to the lunar surface on a variety of landers and rovers. LPX has size and shape of a 1U CubeSat (10 cm on a side) and a total mass of 0.3 kg. The base will contain a seed module holding around 50 *Arabidopsis* seeds and provide ~ 0.5 liter of normal air at standard pressure. By the action of a small pump, water will be released after landing to initiate germination. Images will document the germination, initial growth, phototropism, and circumnutation of the small plants while sunlight remains available. A CO_2 sensor tracks the release of CO_2 during germination and the subsequent uptake of CO_2 by photosynthesis. Simulations indicate that the CO_2 peak level will be an increase by 2250 ppm after 2 earth-days and then decline to about 1000 ppm after 7 earth-days. The camera and CO_2 sensor interfaces are USB. The water pump initiation requires a 5 volt signal. After initiation of germination by the addition of water, the habitat must be maintained above 22ºC to allow for plant growth and below 27ºC to prevent damage to the plants. This relatively tight temperature tolerance will require thermal control systems using reflecting surfaces and insulation that must be specifically designed for each lander or rover. The plant growth unit will function to tilt angles of 20º along any axis. Total image data volume required to gauge the plant growth rate and leaf area is 1Mbyte (minimum data required) to 40 Mbyte (preferred data). The lid of the habitat will allow adequate sunlight for plant growth. To provide for optimum growth, light levels in the plant growth stage need to be between 75 and 150 umole m^{-2} s^{-1} in the 400 to 700 nm interval.

Background:

The importance of plants for long-term life support in human bases on Moon and Mars has long been understood and is of continuing interest. However, there are very little data to show how plants will grow in the partial gravity and high radiation on the surface of the Moon or Mars. The vast literature on the growth of plants on the surface of the Earth has been augmented by a growing literature on plant growth in microgravity. How biology responds to partial gravity remains uncertain. It has been implicitly assumed by the mission planners who have been designing long-term human missions to the Moon and Mars that biological development and response to 1/3 and 1/6 gravity will be more akin to 1 g than to microgravity. Only recently have studies with small centrifuges on ISS allowed for investigations at partial gravity begun to test this assumption.

Major goals:
The plant science goal for LPX is to demonstrate germination and initial growth in lunar gravity and radiation.

The technical goal for LPX is to design a 1U habitat that can provide Earth normal atmosphere, sunlight, and water on the surface of the Moon to support a variety of life sciences experiments.

The expected LPX science results can be listed, in the following priority order, as:
Germination in lunar gravity and radiation
Rate of growth (leaf area index) versus time
Observation of circumnuation
Observation of phototropism
CO_2 release during germination and subsequent uptake by photosynthesis.

Figure 1 shows an image of the plant growth stage with Arabidopsis sprouts after 7 days of grown. Image was taken with the camera in the LPX mockup.

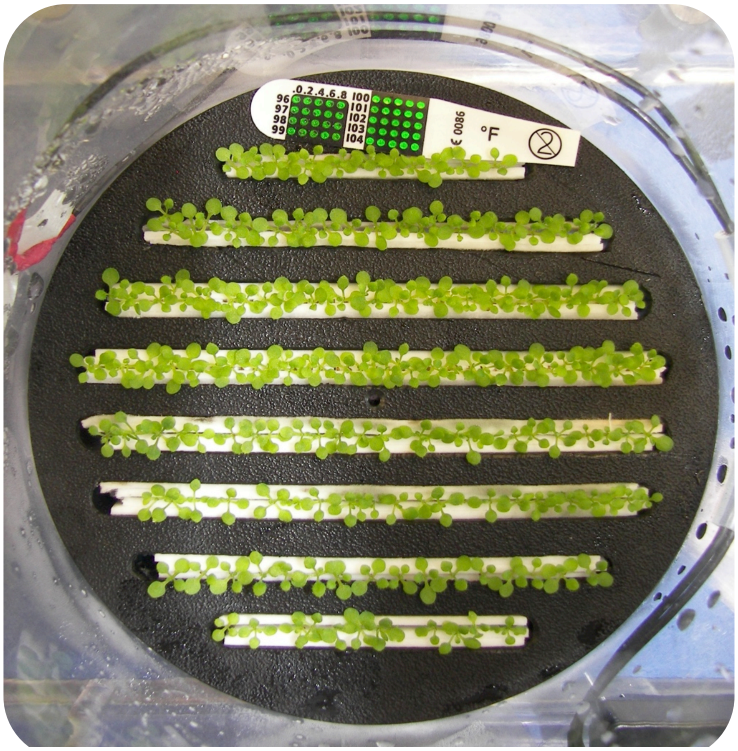

Figure 1. Arabidopsis after 7 days of growth in a sealed chamber. Maximum height is 1.2 cm.

Figure 2 shows data from the growth of 150 seeds of *Arabidopsis* in a sealed 1.3 liter container. We estimate that approximately 50% of the organic C in the seeds was released during germination resulting in the increase of 2250 ppm CO_2.)

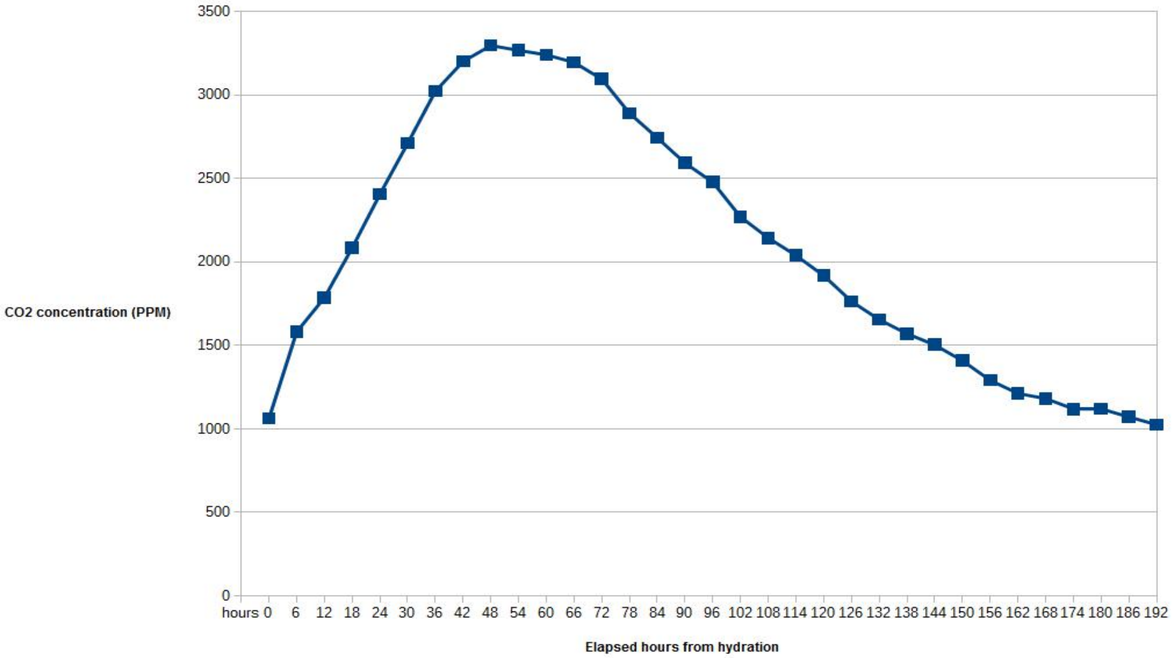

Figure 2. CO_2 over time for 150 seeds in a 1.3 liter closed volume.

Habitat Design and Operation:
We present a preliminary design for the LPX module. The LPX habitat is designed to have minimal impact on the mission design, spacecraft, and lander operations. The design is open and represents an initial engineering design by NASA published in order to allow researchers and university students to modify it to meet their own science/engineering experimental goals. Improvements and augmentations to the basic NASA design will be collected and posted for open sharing.

The habitat is a made out of aluminum with a lexan / polysulfone window on the top. The habitat on the inside contains the seed module to hold seeds mounted on Whatman filter paper using guar gum, the water storage and pump delivery system, camera, CO_2 sensor, and an electrical pass through. All the power, operations and data on the habitat will be controlled by the lander where a cable using suitable interface to attach the habitat and spacecraft will be used (based on the power, data interface requirement of the spacecraft).

Table 1. Main design drivers for LPX.

	Property	Requirement	Description	Notes
1	Shape	Cubesat form	1U Cubesat (10 cm in each dimension)	CubeSat standard
2	Mass	300 grams	Basic weight of the habitat without connectors and interface to the spacecraft	Minimal mass design for small spacecraft.
3	Volume	Internal volume 0.5 liter	Provide a hermetically sealed container with Earth-like atmosphere	Provides more than enough CO_2 for plant growth for the number of seeds
4	Mounting location	Inside or outside the lander		The window should be exposed to receive external sunlight or LEDs added internally. Minimum light requirement is continuous 75 umole m^{-2} s^{-1}.
5	Thermal control	After water addition: 22ºC - 27ºC	Below 22ºC the plants will grow slowly, above 27ºC the plants may germinate erratically.	This is a key mission and spacecraft specific issue and is discussed in a separate section below.
6	Interface	USB	Camera and CO_2 sensor are USB	Emerging standard for electronics simplifies development, testing and provides standardization.
7	Spacecraft accommodation	Spacecraft dependent payload	Payload designed to operate with most spacecraft architectures; all operations will be preprogrammed	Will need spacecraft power and operating capacity to perform trigger for water, camera, CO_2 sensor, data collection and transmission.
8	Flight operations		The lander should be able to run and operate the habitat	Since the flight operations are so simple it is more suitable to use the lander computer to run the habitat
9	Ground operations		No ground operations envisioned other than processing post flight data	No specific ground operations required once the experiment is controlled by the lander computer

Table 2. LPX List of major parts/components.

No	Component	Current Description	Options	Availability
1	Habitat shell	Made machined out of aluminum T6061 alloy	Could be 3D printed in Aluminum and or directly molded in polycarbonate material used for astronaut visor	Machined aluminum available globally. Other two options availability – limited
2	Habitat lid	Aluminum lid with lexan window	Complete lid can be made out of astronaut visor material or lexan	Astronaut visor – limited availability Lexan – available globally
3	Root module	3D printed ABS	Machined delrin could also be used	Both available globally
4	Camera	ELP 2MP Fish eye camera http://www.amazon.com/s/ref=bl_sr_photo?ie=UTF8&fieldbrandtextbin=ELP&node=502394	ELP 2mp or 5MP. Standard or wide angle lens	Available globally
6	CO_2 Sensor	ELT MTG-100 http://eltsensor.co.kr/2012/eng/product/co2-sensor-module-T100.html	ELT T110G-3V	Available globally
7	Water pump	Takasago SDMP 7ML/MIN http://www.takasago-fluidics.com/products_pump/piezo/index.html	Servoflo MP6 http://www.servoflo.com/micropumps/mp6.html	Available globally
8.	Water bag	Smiths 50 ml medical cassette reservoir http://www.smiths-medical.com/catalog/ambulatory-pump-disposables/cadd-disposables/cadd-medication-cassettes/cadd-medication-cassette-reservoirs-1.html#		Available globally
9	Tubing	Tygon http://www.biopharm.saint-gobain.com/en/Products.asp?ID=52		Available globally
10	Electrical pass through	Final pass through will be selected based on spacecraft interface design http://www.srihermetics.com/		Global availability to be confirmed
11	Colorimetric water sensors	3M 5557 3MM-DISC-100 http://www.amazon.com/TapeCase-5557-0-118in-Circle-Indicator/dp/B007Y7II1A/ref=sr_1_5?ie=UTF8&qid=1425948025&sr=8-5&keywords=water+indicator+tape		Available globally

A functional diagram of the 1U CubeSat LPX habitat is shown in Figure 3.

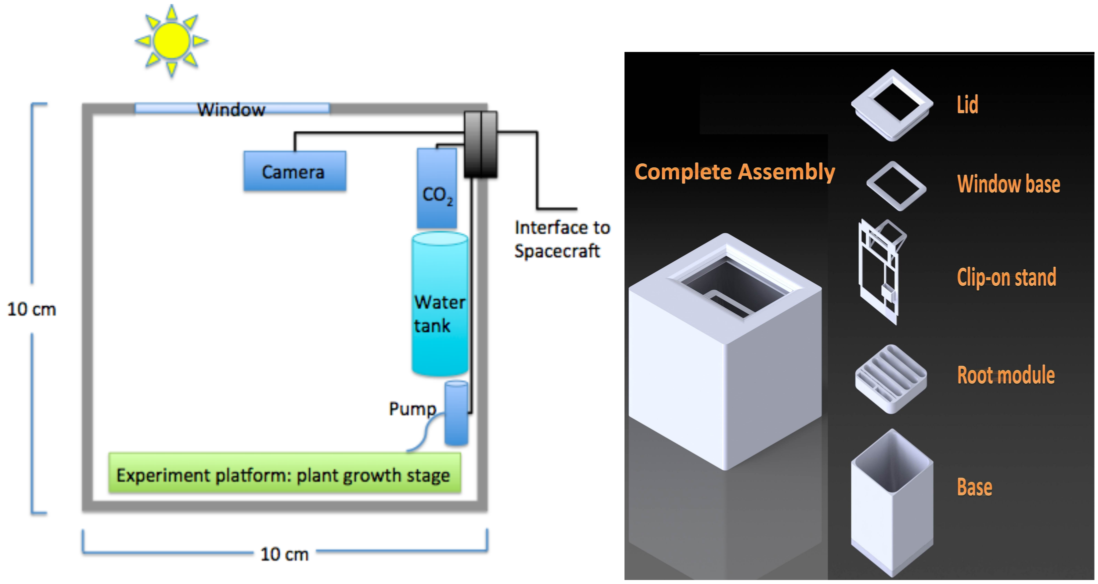

Figure 3. The 1U CubeSat LPX habitat showing the camera, CO_2 sensor, water delivery system, and plant growth stage. In this design there is a window in the top to allow sunlight to enter. Right panel shows a 3D model of the components suitable for 3D printing /machining.

Seeds and seed growth platform:
The primary plant used in our habitat is *Arabidopsis*, used widely in plant physiology experiments on ISS. The seeds will be carried in the seed module at the bottom of the habitat. The module is made from ABS/ Delrin, an insulating and biocompatible material, to help stabilize the temperature of the seeds. There are furrows in the polymer, lined with erect blotter paper and polypropylene combs inserted in rows spaced 0.5 cm apart. This design is similar to shuttle and ISS-heritage plant experiments. The comb provides air to the root system and ensures plants are adequately spaced for science imaging. Over 50 seeds can be accommodated in the LPX habitat. Over the 8 days of the nominal mission it is expected that the plants will reach a height of ~1 cm -1.5 cm. This sets the depth of focus required for the camera.

Camera:
The camera system is a small, high resolution web cam. These are commercially available units. Our basic design is based on a 180 degree fish-eye camera. The 180 degree lens causes geometric distortion of the image but this distortion can be easily corrected with image processing software. This type of lens allows not only to capture the root module but also look at the sides of the habitat where the water reservoir, pump and CO_2 sensor are located. It also allows a depth of focus sufficient to image *Arabidopsis* shoot growth up to 2 cm. The camera is located directly above the growth stage to look down on the plants providing the best view for determining the plant leaf area. The roots are not imaged in the current system. From these images we will be able to determine that germination has occurred, track the leaf area over time and track the position and orientation of individual plants. Ideally, the images will be obtained at a rate of one per

six hours after water is added. However, to document circumnutation, it would be preferable to collect images at 5 minute intervals for at least 6 hours on one of the days (day 5,6, 7). Because the plants grow and move slowly, image compression can greatly reduce the total data volume that must be downloaded to Earth. The images from the camera also are used to determine the water state of the seed platform by use of small disks that change color when wetted.

Water pump, reservoir bag, & colorimetric water sensors:
The water delivery system depends on pump pressure and capillary action to wet all seed module furrows. It consists of a water bag made of biocompatible material and a pump that serves as a valve until it is activated. Once activated it then pumps the water through a tube to a reservoir under the seed module that is packed with blotter paper with a layer of gauze cloth (cheese cloth). The absorbent paper wicks the moisture across the bottom of all seed furrows, which are also filled with blotter paper. The freeze-tolerant water bag has flown in space on NASA - Roscosmos BION experiment (Bion is a 14-30 day Russian LEO biological experiments capsule). The current intended pump is a piezo electric micro pump. During the experiment colorimetric wetness indicators will show the extent to which water has wetted the seed module. This material turns red when exposed to liquid water and provides a way to use our images to confirm water delivery. This indicator tape has been tested for biocompatibility, freeze-thermal and vibration adhesion. Small disks that change color when wetted are placed in several locations on the plant growth stage.

Passive pressure indicator:
We will use a small piece of biocompatible plastic bubble wrap as a passive pressure indicator to detect total loss of pressure due to a leak.

Container and seals:
The habitat can be made of standard aerospace grade Aluminum alloy T6061 for good thermal control. The habitat can also be 3D printed using aluminum powder or be made out of coated polysulphone /lexan. The lid of the container could be made of aluminum or a clear lexan panel or a combination of both- basically a lexan window in an aluminum lid. For the current design the habitat will be machined from an aluminum block with a lid and window top. Seals (O rings) used will be of the same material and tolerances as those used on similar previous experiments flown in space. An electrical pass-through will allow the lander to communicate with the habitat, initiating the sensor suite, and download imaging data. The final electrical pass-through would be selected based on the spacecraft interface requirements.

Payload/Instrument Integration/Accommodation/Location Requirements:
There are a few outline requirements to accommodate the payload with the lander. First, the habitat interior should be maintained between 22ºC and 27ºC and must not reach temperatures much over 27ºC during the lunar day. Secondly, the habitat must be electrically connected to the lander computer for control and data transfer. Thirdly, the habitat should be placed such that is can receive natural sunlight (exposing the top window). There are two possible mounting locations: 1) On an external surface which

will provide the best exposure; 2) Inside the lander and exposing the top window to sunlight. An alternative to use of sunlight might be inclusion of internal LEDs to provide sufficient light for germination and seedling growth. Use of internal LEDs provides sufficient lighting to insure experiment success, regardless of habitat position or window exposure to sunlight.

Payload Operations:
Following is the sequence of operation for the habitat.
The habitat can be assembled and seeds put in place a month or more before the flight (preferably – as close as possible to flight date).
Once mounted the seeds stay dry and dormant during launch and flight to the surface of the moon. During this period the habitat does not require any power or data stream from the spacecraft. The seeds can survive temperature excursions from -160º-to 55ºC.
Once on the surface on moon with lander ready to start experiments, this experiment will be initiated by obtaining a baseline CO_2 reading, a reference image, and then activating the water pump to initiate germination.
CO_2 readings and images are then obtained as often as the data system can be accommodated, ideally once every 6 hours as described above.
The CO_2 reading and camera images are stored on board the spacecraft until offloaded by the computer.
All data is downlinked to Earth for analysis by the Science Team (It is not anticipated that the team will send commands to the habitat).

In-Flight Calibration and Testing:
There are no inflight calibration and testing requirements.

Data Plan:
All raw data, calibration records, and processed data from LPX will be maintained in an updated form throughout the period of investigation. A continually updated record of the "best version" of the data will be maintained until meaningful changes in data calibration no longer occur.

Data Reduction and Validation:
All images and data will be released to the public as they are downloaded and calibrated (within 24 hours). All data will be placed in the Planetary Data system within 30 days of data download. The final archival data will be stored in the Planetary Data System (PDS) within 90 days after download. Images will be processed by the Science Team and plant height and leaf area will be determined on ground. The imaging sequences for growth, development, phototropism and circumnutation will be assembled into videos showing these characteristic plant motions. A publication of these results will be submitted within 90 days of complete data download.

Performance Margins:
The key performance requirement is on the camera. The baseline camera design use has a performance margin of at least a factor of 2. This means that the resolution of the images

is 2 times the resolution needed for our science goals. This margin is maintained in the event of data bit dropout due to radiation effects.

CO_2 sensor:
The CO_2 sensor is a commercially available unit that is small, USB operated, and reads over a range of CO_2 from 0 to 10,000 ppm ±50 ppm.

Minimal Data:
One quality image at day 5-8 is adequate to confirm if germination and early growth occurred. The record of CO_2 provides an independent indication of germination and photosynthesis.

Preferred Data:
Color images of the plant growth area and CO_2 readings every 6 hrs (or less) for the full duration following hydration. If not possible, then one image per day following hydration (to verify hydration event) followed by images every 6 hrs commencing with day 3.

Data plan rationale:
Minimal image data confirms that environmental conditions were sufficient for germination and early growth, resulting in photosynthetic tissues capable of generating O_2. The preferred data provide for assessment of detailed plant growth including rates of germination and growth. Six-hour frequency of daytime images will demonstrate phototropism as the angle of incident light changes through the lunar day. A burst of images taken at 5 minute intervals, taken on approximately day 7 in late afternoon, for approximately 4-6 hours to document circumnutation. Images can be recorded and downlinked later as bandwidth permits. Detection of all of these earth-normal growth parameters will allow for detailed comparison of plant performance under earth conditions.

Instrument Test and Verifications Plan:
The standard collection of tests performed on flight instruments will be applied to the LPX habitat: thermal, vacuum, shock and vibration, extreme temperatures, etc. One test specific to life sciences experiments is extended biocompatibility tests of all habitat material to simulate the longest launch preparation, lunar transit stage, and landed mission (approximately 2 months).

Earth-based and ISS controls:
The scientific results of LPX must be compared to Earth-based controls and ISS microgravity, and partial gravity experiments. The 1U CubeSat design allows for easy replication by students and researchers on Earth and for accommodation on ISS.

Additional sensors:
The basic LPX has only a CO_2 sensor and a camera. However additional sensors could be considered in variants or future designs. These include:
External temperature
Ionizing radiation
Internal temperature

Internal light level, intensity and spectrum
Internal RH
Pressure
O2 partial pressure
Ethylene absorbent

Thermal Control:
A key requirement for the success of LPX is temperature control. The range of temperature in the habitat after initiation of growth must be between 22ºC and 27ºC - a much narrower range of temperature than typical for flight electronics or flight instruments. This poses significant challenges. The general approach that is to design the LPX surface coatings and position on the lander such that the equilibrium temperature is ~20ºC and then to add a small root module heater to bring the temperature up to desired range.

The design of reflective and heat emitting surfaces and their coupling to the LPX module will depend on the landing latitude of mission. For missions equatorward of 45º the challenge will likely be keeping the habitat cool. For missions which land poleward of 45º, the challenge will likely be keeping the habitat warm. A significant variable in this problem is the extent to which the habitat is warmed by the lunar surface and light reflected from the spacecraft surfaces.

This aspect of the LPX design will require considerable work specific to the mission architecture and the chosen landing site and hence can only be done after these are specified. Inclusion of an internal thermocouple and root module feedback-controlled heater greatly reduces reliance on external surfaces to maintain a biologically appropriate internal temperature.