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Development of Equipment that Uses Far-Red Light to Impose Seed Dormancy in Arabidopsis for Spaceflight

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ABSTRACT

In order to use plants as part of a bioregenerative life support system capable of sustaining long-term human habitation in space, it is critical to understand how plants adapt to the stresses associated with extended growth in spaceflight. Optimally, dormant seeds would be germinated on orbit to divorce the effects of spaceflight from the one-time stresses of launch. At an operational level, it is also important to develop experiment protocols that are flexible in timing so they can adapt to crew schedules and unexpected flight-related delays. *Arabidopsis thaliana* is widely used for investigating the molecular responses of plants to spaceflight. Here we describe the development of a far-red light seed treatment device that suppresses germination of Arabidopsis seeds for periods of ≥12 weeks. Germination can then be induced when the seeds encounter red light, such as transfer to the illumination from on orbit plant growth hardware. This device allows for up to twelve 10×10 cm square Petri dishes containing seeds on nutrient gel to be irradiated simultaneously. The far-red device is contained within a light-proof fabric tent allowing the user to wrap the Petri dishes in aluminum foil in the dark, preventing room lights from reversing the far-red treatment. Long-term storage of the wrapped plates is accomplished using foil storage bags. The throughput of this device facilitates robust, high-replicate biological experiment design, while providing the long-term pre-experiment storage required for maximum mission flexibility.

Key words: Seed Dormancy; Orbital; Plants; Phytochrome; Spaceflight; Far-red Light

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INTRODUCTION

One key element being explored to support a sustained human presence in space is the feasibility of a bioregenerative life support system to sustain astronaut health. Bioregenerative life support provides air and water purification, produces food, and allows waste management through the use of plants and microbes (e.g.,

Lasseur et al., 2010; Paradiso et al., 2014). An understanding of how plants respond to the spaceflight environment will be critical to defining the biological and engineering challenges in having them thrive in Earth orbit and beyond (e.g., Kiss, 2014; Richards et al., 2006; Kittang et al., 2014; Paul and Ferl, 2015). Such research requires monitoring plant physiology, growth, and development in space, and has used platforms including sounding rockets, parabolic flights, Space Shuttle missions, and, most recently, analysis of plant growth on board the International Space Station (ISS; e.g., Paul and Ferl, 2015; Paul et al., 2013; Vandenbrink and Kiss, 2016). Historically, pre-grown plants were launched into orbit, grown, and returned for analysis. However, interpretation from these experiments is confounded by the effects of the increased G-forces and stresses experienced during launch and landing. These issues are now overcome by launching seeds, followed by germination on orbit. Grown plants are harvested while still in microgravity and then chemically fixed and/or frozen for sample return to Earth for further analysis.

Although ISS plant growth facilities such as the Vegetable Production System (Massa et al., 2013) and the European Modular Cultivation System (Correll et al., 2013) allow germination of dry seeds following activation by watering, basic plant spaceflight research also uses a simplified scheme whereby seeds are planted on nutrient gel (usually in Petri plates) under sterile conditions prior to launch and transport to orbit (e.g., Paul et al., 2012a,b; Kwon et al., 2015). Although this approach provides a robust experimental design to planting the seeds and providing nutrients for subsequent growth, a key problem with this strategy is that the seeds of many species of plants, including the most widely used model plant *Arabidopsis thaliana*, germinate within only a few days of imbibition, as inevitably occurs when planted on gel nutrient media. Germination can be delayed by several days by storage of the planted plates at 4˚C and such cold treatment is a currently used strategy. However, even at these lowered temperatures, germination eventually occurs at around day 10 following setup on Earth, providing little safety margin for unexpected delays in launch or on orbit operations.

One further strategy to delay germination is to capitalize upon the normal regulatory mechanisms controlling seed germination. For example, Arabidopsis germination is promoted by light, and so maintaining seeds in dark conditions can help prevent premature germination for several days (e.g., Paul et al., 2012a,b). Further, in many plants, germination is inhibited by far-red light (~740 nm) and this inhibition (dormancy) is reversed by subsequent irradiation by red light (~660 nm; e.g., Borthwick et al., 1954; Shinomura et al., 1994; Oh et al., 2004). The molecular machinery behind this red/far-red reversibility is well understood and aids in the practicality of using it in an approach to delay germination for flight. Thus, genes that produce the germination-inducing plant hormone gibberellin (GA) are upregulated within 12-24 hours of seed imbibition (Yamaguchi et al., 1998), leading to germination within a few days of taking up water. Far-red light triggers the photoreceptor phytochrome to repress the GA biosynthetic machinery and signaling system (Toyomasu et al., 1998; Yamaguchi et al., 1998; Oh et al., 2004, 2006, 2007), in addition to enhancing the stability of other inhibitors of GA action (such as DELLA proteins and the hormone abscisic acid; Piskurewicz et al., 2009; Seo et al., 2006). Thus, far-red irradiated seeds are dormant even though they are imbibed. This molecular network controlling germination has the practical effect that Arabidopsis seeds irradiated with far-red light become dormant even when well-watered, providing they are not exposed to red light after the far-red irradiation. Germination can then be triggered by providing a red light stimulus, such as from the illumination of the plant growth hardware on orbit. This biological phenomenon gives researchers a mechanism to plant seeds under optimal germination conditions on the ground (well-watered, high nutrient media) yet prevent seed germination in a controlled manner, allowing time for both transport to the ISS and flexibility to cope with possible delays in on-orbit activities prior to seedling growth.

The use of far-red light treatment to induce dormancy for spaceflight experiments was initially reported by Nakashima et al. (2014) as part of the protocol for the APEX-02 Arabidopsis growth experiment on the ISS, using a combination of 10 min far-red irradiation and cold

stowage to delay germination. We report here the design and implementation of second generation equipment for inducing dormancy with far-red light that greatly increases throughput by allowing parallel processing of 12 samples. The equipment is designed to be scalable and to operate with the minimum of laboratory requirements, making setup feasible at launch facilities with limited wet-lab capabilities.

METHODS

Prototyping

Three design requirements drove initial prototype design for this far-red irradiation device. First, an LED-based system was needed to provide the specific far-red irradiation wavelength with a mechanically robust design. Second, relatively high powered LEDs were required for high irradiation intensity, potentially reducing the time needed for far-red treatment. Far-red inhibition of germination shows reciprocity, where longer periods of lower fluence can be mimicked by higher intensity light of a shorter duration (Shinomura et al., 1996). Third, a high throughput method was needed by which several 10×10 cm square Petri dishes could be irradiated in one time period.

XP-E far-red LEDs (peak wavelengths 720-740 nm; Cree Inc., Durham, NC) form the core of the equipment, running at 700 mA and 2.1 VDC. An initial design was created to test if a minimal engineering solution was feasible, or whether a more complex equipment design would be required. The first prototype was also used to define major potential design issues with using the XP-E LEDs that would require solutions. To this end, irradiation with a single LED held on a simple 3D printed mount laying on the surface of a 10×10 cm square Petri dish containing seeds was used, as shown in Figure 1. The design was modeled in SolidWorks (Dassault Systèmes SolidWorks Corporation, Waltham, MA) and printed on a Makerbot Replicator 2 (Makerbot Industries, New York, NY) using polylactic acid filament. The LED in its mount on the Petri dish was placed within a lightproof Mylar™ bag (6”×8”×2”, USA Emergency Supply, Colfax, NC). The wires connected to the LED provided a convenient tether with which to remove the LED from the bag after the required period of irradiation, without exposing the now far-red treated seeds inside the dish to room light. Once the LED rig had been extracted, the bags were then Ziploc sealed and heat sealed using a V201 heat sealer (Ziploc, Racine, WI).

During testing of this first prototype, it was discovered that the XP-E LEDs generate enough heat to approach temperatures known to trigger thermal stress in Arabidopsis (Saidi et al., 2011), with air temperature close to the Petri dish rising to 32.5˚C and the surface temperature of the LEDs rising to 68.0˚C. In an attempt to remedy this problem of heat buildup, a second prototype was developed using the same 3D printer approach, but with an open pyramidal design to promote convective cooling (Figure 1c-e). This mount centered the LED over the Petri dish in the same manner as the initial holder, but did so while allowing more airflow and ventilation for passive heat dissipation. Even with this ‘pyramid’ design, the far-red LEDs led to equivalent heat buildup as with the first holder design. This initial prototyping defined the incorporation of a larger air volume around the irradiated dishes, coupled with the use of a heat sink and potentially an active cooling system, as likely critical design features to make an effective irradiation system using these XP-E LEDs.

Final Design

The final design is comprised of two major parts: (1) a PVC light-proof tent frame with a fabric canopy of blackout material providing the large working volume for the far-red irradiator to work within, and (2) an actively cooled, LED-based far-red light emitter. A parts list for the device is presented in Table 1.

Blackout fabric canopy and PVC frame

The previous published design for a single LED far-red illumination box (Nakashima et al., 2014) had required its use in a dark room. This was due to the fact that, while the LED irradiator was in a light-tight box, plates had to be removed from the equipment post-irradiation and wrapped in foil to maintain the inhibition of germination imposed by the far-red light. To allow more versatility in lab setting, the design described herein makes use of a custom benchtop light-proof tent in which all steps from irradiation to wrapping can be performed even in ambient room light. Thus,

supplies for wrapping and storing the irradiated Petri dishes (aluminum foil and foil storage bags), samples, and the far-red light emitter all sit inside the blackout tent and are manipulated in a similar manner to a laboratory glove box. A user inserts their hands into long sleeves to access the inside of the tent. A USB Webcam camera (LE, 20M Pixel) allows monitoring of the internal area, facilitating manipulations such as wrapping each irradiated plate in foil.

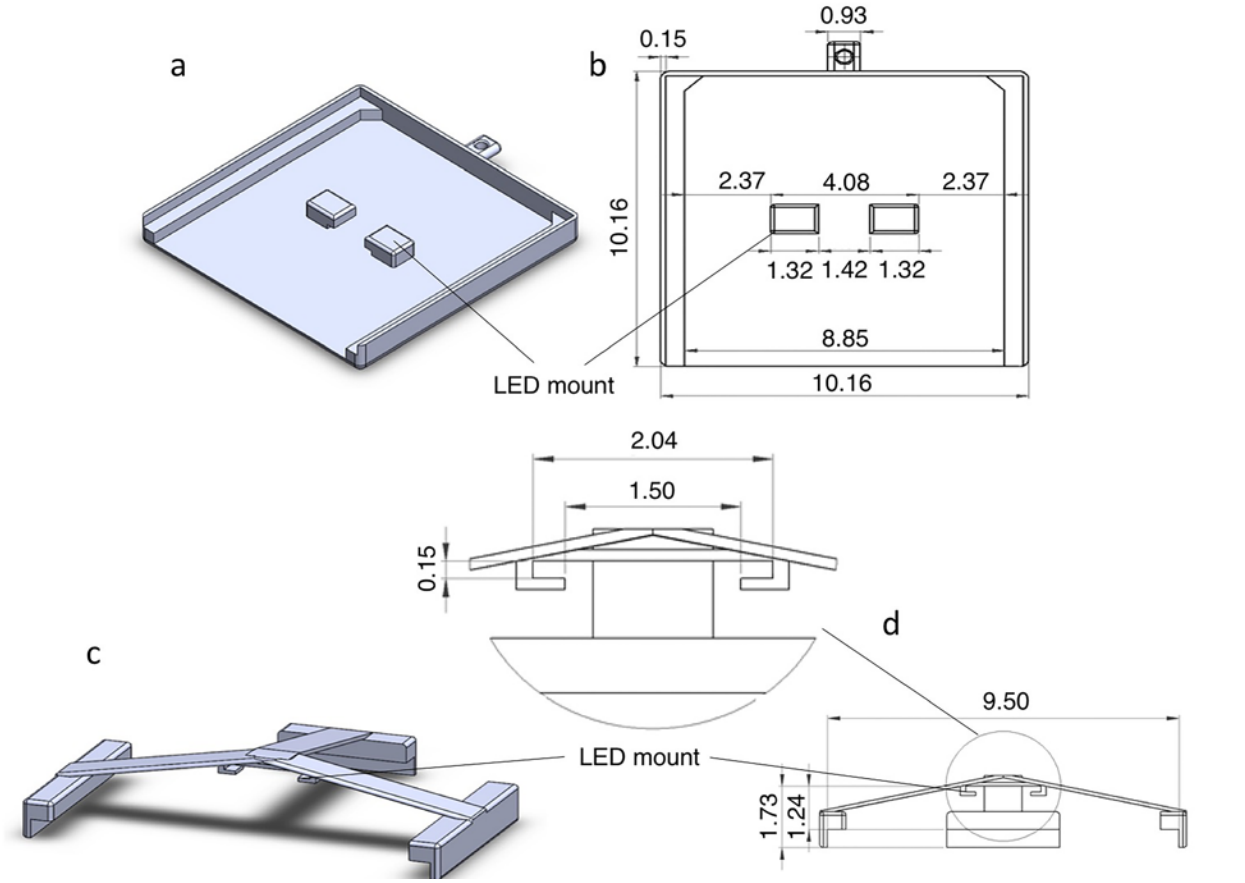

Figure 1. (a-b) Initial prototype Petri dish cover design holds a single far-red LED over the center of a 10 cm×10 cm Petri dish using the integral clips. Wiring from the LED wrapped through the tab in the back allowing for a user to remove the entire LED assembly from a Mylar bag that was used to provide a dark environment for the Petri dish after irradiation. (c-d) Second prototype 'pyramid' design that held a single far-red LED over the center of a 10 cm×10 cm Petri dish, but allowed for increased convective air flow. All dimensions are in cm.

This desktop light-tight tent is comprised of a frame of ½" PVC tubing (J M Manufacturing, Wilton, IA) and blackout fabric (Thorlabs Inc., Newton, NJ). The fabric is cut into two sheets, such that one is used for the front and one for the rest of the cube. The PVC frame is one rectangular prism, with an additional frame element at the front for the sleeves as shown in Figure 2a. The PVC supports also act as a placement for Velcro to hold the blackout fabric taught. The front supports allow for two sleeves to be attached to the tent. These sleeves are mounted through two 4" diameter plastic rings that themselves are bolted to the PVC frame. This support is required to prevent users from ripping the front light-proof fabric, since testing indicated that once their arms were inserted into the sleeves, users inevitably lean on the frame and fabric during operation of the equipment, leading to tearing of unsupported parts of the sleeve/tent junction. Sleeves were attached to two holes cut into the front tent panel using Velcro and foil tape (322, Nashua Corp., Nashua, NH).

Table 1. Parts list for the light rail, tent, and frame.

Light Rail			
Qty	**Item**	**Qty**	**Item**
4	24” Fractional 10 Series 80/20 (80/20 Inc.)	3	3/32” × ½” × 36” A2 Air Hardening Ground Flat Stock (Drill Rod & Tool Steels, Inc., Franklin Park, IL)
4	6” Fractional 10 Series 80/20	2	Constant Current LED Drivers 700 mA (RapidLED)
6	10 Series 2 Hole - Inside Corner Bracket 80/20	8	Barbed Hose Fittings
6	10 Series 4 Hole - 90 Degree Angled Flat Plate 80/20	2	Plugs without grounding prong
40	XP-E Far-red LED (Cree Inc.)	1	Dynamax 412 Pump, 12 V (Simply Pumps, Manor, PA)
80	1/4”-20 × 5/16” Machine Screws	1	½ Gallon Reservoir
N/A	¼” Silicone airline tubing (Petsmart, Phoenix, AZ)	N/A	60/40 Rosin Core Solder
N/A	Silicone Heat Sink Compound (Synco Corp., Bohemia, NY)	N/A	22 gauge hookup wire
N/A	PTFE Tape		
Tent and Frame			
1	Blackout Fabric with Rubberized Coating (Thorlabs Inc.)	N/A	½” Schedule 40 PVC pipe (J M Manufacturing, Wilton, IA)
16	½” Schedule 40 Tee Fittings (Dura Plastics, Beaumont, CA)	8	½” Schedule 40 Corner Fittings (J M Manufacturing)
2	Flange Extension ring (Sioux Chief, Peculiar, MO)	N/A	322 Foil Tape (Nashua Corp., Nashua, NH)
N/A	2” Industrial Grade Velcro (Velcro Industries, Manchester, NH)	N/A	1” Velcro (Velcro Industries)

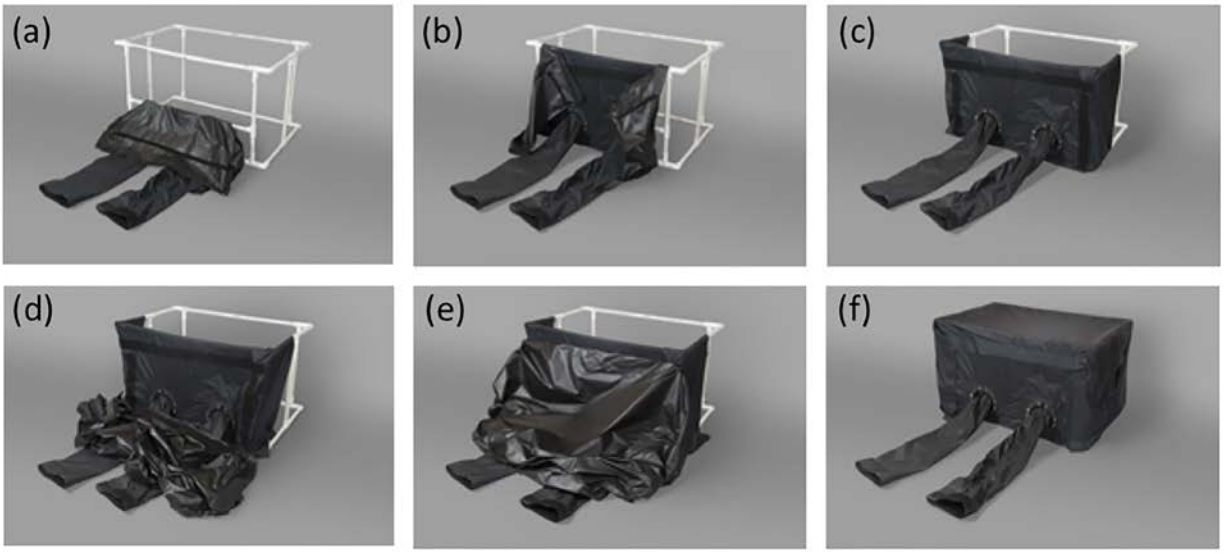

Figure 2. (a-f) Assembly of the portable benchtop darkroom. The tent covers the PVC frame with integral sleeves to allow access to the Petri dishes to be irradiated inside without interference from ambient room lights. Dimensions are 93 cm×62 cm×62 cm.

In order to assemble the canopy of the tent, the front piece of fabric is wrapped around the user-facing side and the top bar is then Velcroed to itself as shown in Figure 2a. The front piece of blackout fabric is pulled taught around the left and right corners and Velcroed to the top right and left PVC bars as shown in Figures 2b and 2c. The top panel is then Velcroed to the front panel (Figures 2d and 2e). Pulling the top panel taught, the user can then fold the right and left panels around to Velcro onto the front piece of fabric as shown in Figure 2f. Access is available from the back of the frame, as the blackout material at the back is not attached to the PVC in this region. Once all materials for the experiment are loaded into the tent, the back is draped down the frame with the extra laying on the lab bench and making a light-tight seal to the bench surface.

Far-red light rail

For irradiation, Petri dishes sit beneath an array of 40 far-red LEDs delivering 18 µmol/m^2/s at the Petri dish surface. The design consists of four light rails of 25.4×25.4 mm 80/20 (four open T-slots profile; 80/20 Inc., Columbia City, IN), each with 10 far-red LEDs mounted evenly spaced and staggered with respect to the neighboring light rail to improve light coverage as shown in Figures 3a-c. The LEDs are wired as two independent circuits of 20, run in series at 44 VDC and 700 mA from a constant current driver (LPC-35-700, RapidLED, Burlingame, CA). Each LED has a custom steel nut as shown in Figure 3d, built to fit the exact size of the LED. The nut mounts the LED to the 80/20 rail and thermal grease is used to facilitate a higher rate of heat transfer from the LED to rail.

Cooling system for LEDs

Because the initial prototyping described earlier had indicated that the LEDs can drive local temperatures well above the safe operating levels for the seeds to be irradiated, a liquid cooling system was built into the 80/20 rails. Each 80/20 rail has a hollow core and has had the center hole on each end tapped to 19.05 mm diameter to take a 20 threads per 25.4 mm fitting. In each hole, a barbed hose fitting is inserted and 6.35 mm diameter silicone tubing attached to each, creating one long snaking pipe. The inlet and outlet lines for the water supply run to a liquid coolant reservoir sitting outside the darkroom tent. A submersible pump (Dynamax 412, Simply Pumps, Manor, PA) sits in the bottom of the reservoir and pushes water through the 80/20 rails at 1.08 L/min. The outlet ends in the same reservoir, maintaining a constant level of water in the coolant tank. Deionized water is used as the

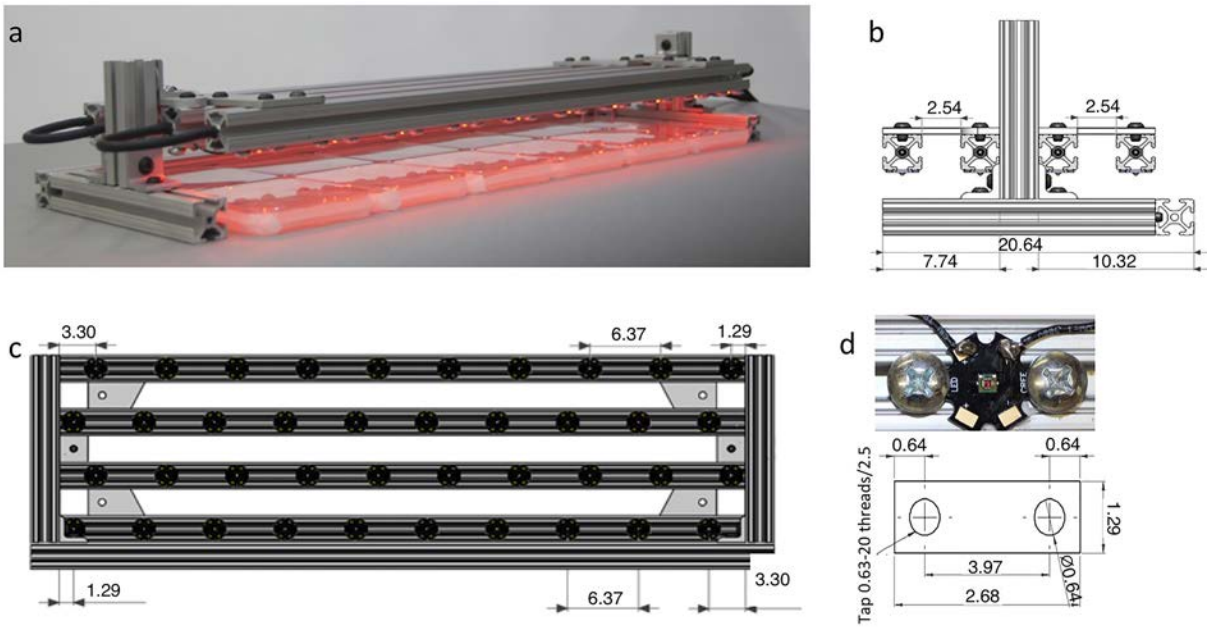

Figure 3. Far-red illumination system (a), side view (b), bottom view showing the alternating alignment of the LEDs (c), and mounted LED viewed from under side and custom steel nut (d). CAD drawings for the extruded aluminum and connectors are from the manufacturer. Dimensions in cm unless noted otherwise.

circulating coolant and extra cooling is enabled by placing the reservoir in an insulated ice bath. When running for extended periods of time (>1 h) additional ice may be needed to keep the whole system cold. With the cooling system running, temperature within the enclosed light-proof tent and the surface temperature of the Petri dishes are maintained close to ambient room temperature (see results section below).

Engineering Testing

To assess possible maximal local heating effects of running the LEDs and robustness of the system to a cooling system failure during use, a series of stress tests were performed where the top and sides of the far-red light rail were enclosed in aluminum foil to reduce heat loss to the surrounding air. A data logger (HOBO, Onset Computer Corporation, Bourne, MA) was placed in the center underneath the light bank recording every ten seconds. As shown in Figure 4, for the first 40 min, the far-red light rail was turned on without any liquid cooling. After the 40 min mark, the liquid cooling system was turned on and allowed to run for another 40 min. The water in the reservoir was chilled in an ice bath before being pumped through the rails. To assess temperature effects on the plant samples under operational conditions, the device was also tested inside the sealed dark room tent and temperature at the surface of the Petri dishes placed under the LEDs measured over 80 min of continuous running with the pump circulating liquid from a cooled reservoir as shown in Figure 4.

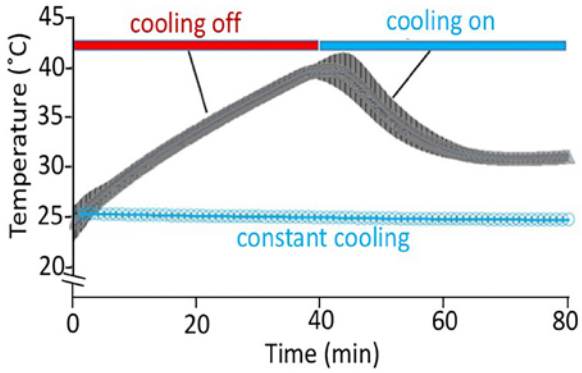

Figure 4. Temperature profiles recorded below the far-red light treatment device. Data represent mean ± SEM, n=3. Upper trace, stress test for local heat buildup, with the liquid cooling system being activated at 40 min. Lower trace, temperature below the light rail with the liquid cooling system running continuously.

Biological Testing

Ten surface sterilized Arabidopsis seeds (Col-0 ecotype) were planted per 10×10 cm square Petri dish containing 35 mL of solidified sterile nutrient gel media (1% (w/v) Phytagel®, 0.3% (w/v) sucrose, and half strength Epstein's plant growth nutrient solution), as previously described (Wymer et al., 1997). Surface sterilized seeds were treated with far-red light either immediately after plating onto medium, or after storage in the dar at 4˚C for 24 h. This latter approach was implemented to test how flexible in timing the use of the technique could be made. In all experiments, Petri dishes containing seeds on nutrient gel medium were irradiated with far-red light for up to 30 min as indicated, and then wrapped in aluminum foil inside the darkroom tent described above. The dishes were inserted into foil storage bags (#6288, ImmunoChemistry Technologies, LLC), which were then sealed outside the tent and stored at either room temperature or 4˚C. lates were unwrapped at the times indicated and the number of germinated seedlings recorded. Germination was defined as a seed with a visible emerging embryonic root (radicle), and was monitored under a Stemi SR dissecting microscope (Zeiss, Thornwood, NY) or Quanta scanning electron microscope (FEI, Hillsboro, OR). The plates were then placed in a plant growth chamber under "white light" delivered from GE-F1518-PL/AQ fluorescent bulbs (22˚C, 16 h day at 80 µmol/m^2/s, 8 h night; Diurnal Growth Chamber #3740; ThermoForma, Verona, WI). Growth was assessed at 7 d later to monitor viability of the samples.

RESULTS AND DISCUSSION

Engineering Testing Results

Figure 4 shows the temperature profiles recorded below the far-red light treatment device during stress testing and normal operation. Over 40 min of a stress test running the LEDs without the cooling system being activated and with minimal convective cooling (by covering the light rail with an aluminum foil tent), temperature around the light rail rose at 0.066˚C s (R^2=0.8826), plateauing at approximately 40˚C (i.e., below temperatures that would cause damage to the hardware but above the point where heat stress to the biology may be initiated). Activation of the liquid cooling system reduced this temperature to a stable 31±1˚C, indicating that the liquid cooling could effectively reduce the heat load generated by the lighting system, even when challenged with limited heat dissipation through other mechanisms. Further testing under normal operational conditions (i.e., no aluminum foil covering) indicated that the cooling system maintained temperatures under the light rail of 25˚C for at least 80 min (Figure 4, lower trace). Thus, when used inside the benchtop darkroom in this actively cooled mode, the air temperature in the darkroom tent remains near ambient, indicating that the liquid cooling system is sufficient to maintain temperatures below those likely to induce heat stress in the seeds being irradiated.

Seed Dormancy and Germination

To test the efficiency of the device at inducing dormancy, plates containing seeds were irradiated for between 1 s and 10 min, wrapped in foil, and stored at room temperature in the dark for 3 weeks. Upon unwrapping, the percentage germination was scored and then the plates were transferred to a plant incubator. Growth after 7 days of white light irradiation was scored as a measure of viability. Table 2 shows that a duration of far-red irradiation as short as 1 s was sufficient to inhibit germination over this period. Additionally, pre-imbibing the seeds for 24 h prior to irradiation had no significant deleterious effect ($p>0.05$, t-test) on suppression of germination (Table 2). The rate of germination was independent of the length of irradiation for both pre-imbibed and non-pre-imbibed samples ($p>0.05$, chi squared), and none of the far-red irradiation schemes significantly affected the viability of the seeds tested (Table 2).

To ensure that such far-red irradiation imposed dormancy did not alter subsequent growth and development of seedlings once the seeds were triggered to germinate, we next imposed dormancy with far-red light. Seeds were kept dormant in the dark for various periods and then seed morphology and subsequent seedling growth were assessed. Figure 5a shows that at the resolution of the scanning electron microscope, there was no obvious change in seed morphology or evidence of germination over the period of far-

Table 2. Effect of far-red irradiation on delaying germination and subsequent viability following unwrapping of plates and germination under growth chamber lights. Plates were irradiated and then stored at room temperature in the dark for 21 days. Results represent mean ± SEM, n≥4. *Percentage of seeds germinated before exposure to growth chamber lights. **Percentage of seeds germinated/grown after 7 days in the growth chamber. n.d., not determined. All germination rates are significantly different from their non-irradiated controls but are not significantly different from each other (p>0.05, t-test). None of the viability scores are significantly different from each other (p>0.05, t-test).

Irradiation	No Imbibition		24 h Pre-Imbibition at 4˚C	
(sec)	Germination (%)*	Viability (%)**	Germination (%)*	Viability (%)**
0	92.5 ± 5	92.5 ± 5.00	95 ± 5.77	95 ± 5.77
1	0 ± 0	100 ± 0	n.d.	n.d.
5	0 ± 0	100 ± 0	n.d.	n.d.
10	0 ± 0	100 ± 0	n.d.	n.d.
30	0 ± 0	96.77 ± 0	n.d.	n.d.
60	2.5 ± 5	100 ± 0	2.5 ± 5	100 ± 0
300	2.5 ± 5	100 ± 0	0 ± 0	100 ± 0
600	0 ± 0	88 ± 3.46	0 ± 0	100 ± 0
3600	2.5 ± 5	100 ± 0	0 ± 0	100 ± 0

red-induced dormancy and storage in the dark. Similarly, when growth was followed after induction of germination, seedling morphology (Figure 5b) and growth rates (Figure 5c) were similar between samples irrespective of the time they were held dormant before induction of germination. These results indicate that far-red irradiation with subsequent storage at room temperature in the dark (foil wrapped plates) is an effective way to delay germination compatible with flight timelines and potential flight-related delays.

Combining Far-Red-Induced Dormancy with Storage at 4˚C

An alternative approach to achieve similar ends for the trip to orbit is storage at 4˚C (although as noted earlier, cold delay in germination may be effective for a more limited duration). Combining these two approaches would have the operational advantage of using two independent methods to delay germination, and so provide a robust, redundant approach to this element of experimental design. However, previous reports have shown a potential interaction between cold storage and far-red treatment (Nakashima et al., 2014). We tested whether combining far-red irradiation and subsequent cold storage using the current device presented any complications when using the Col-0 Arabidopsis ecotype. Analysis of this approach for application to a flight scenario was performed as part of the Science and the Experiment Verification tests for the APEX-05 flight experiment (Gilroy, 2016). These analyses were run at the Kennedy Space Center and mimicked a flight scenario requiring 10 days of dormancy, covering a likely period from the prelaunch handover of samples, to installation in growth facilities on the ISS. Each experiment used 10 min of far-red irradiation, wrapping the irradiated plates in foil and then storage at 4˚C for 10 days. Plates were then unwrapped and transferred to the Veggie plant growth hardware (Massa et al., 2013) running in the ISS environmental simulator. Viability was assessed from the proportion of seedlings germinated and growing after 8 days in the Veggie. These experiments showed that using this approach, the samples exhibited 0% germination and 100% viability (Science Verification test, mean ± s.d. n=5) and 0% germination, 94.5±8.7% viability (Experiment Verification test, mean ± s.d. n=5). Additional testing under identical irradiation and storage conditions performed at the University of Wisconsin-Madison showed samples maintained

0% germination with 97.7±4.5% viability for at least 84 days (mean ± s.d. n=5). Thus, combination of far-red irradiation with cold storage provides a redundant approach to delaying germination compatible with this equipment, although it will be important to rigorously test potential interactions between plant genotype and cold/far-red germination delay for each specific experiment application.

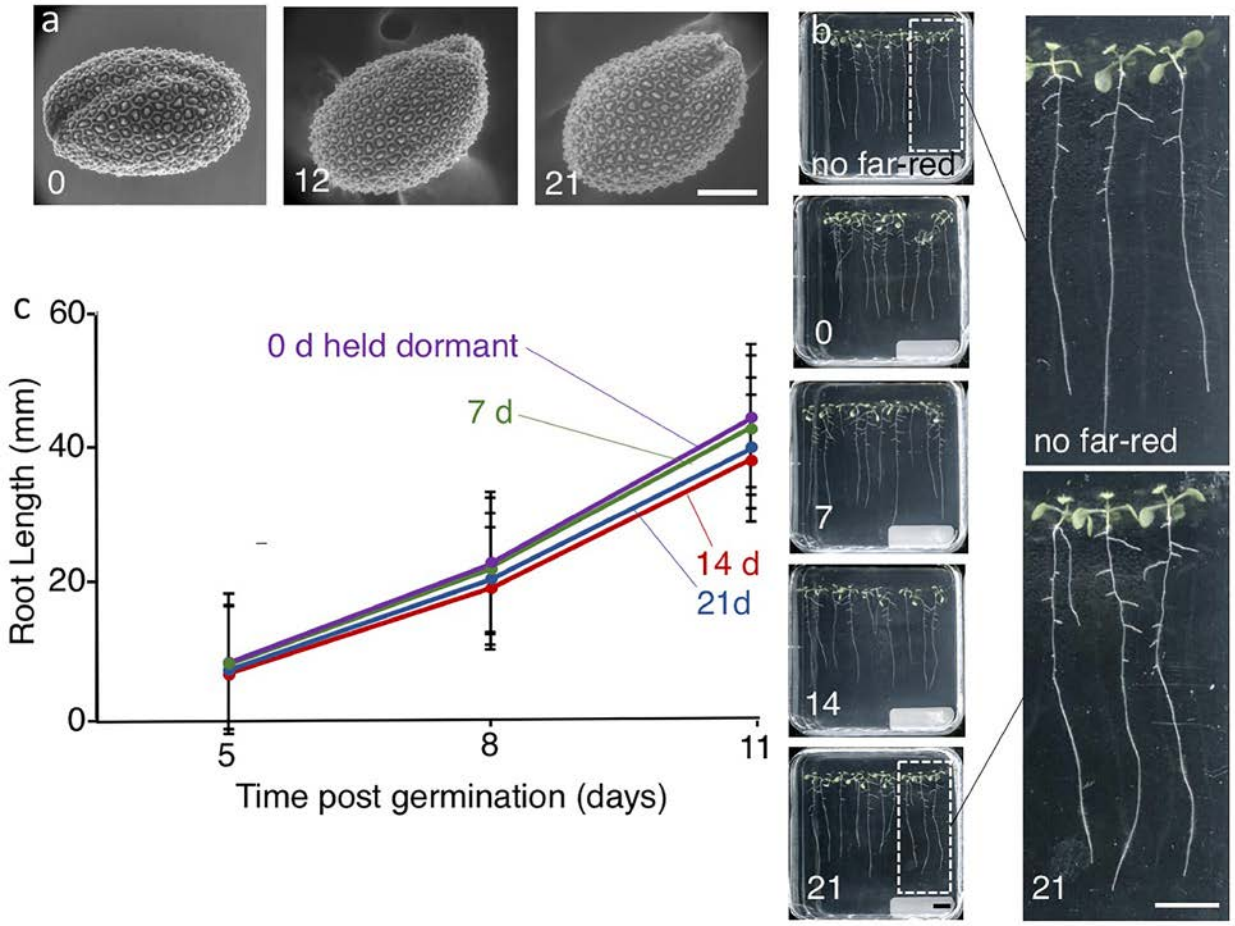

Figure 5. (a) Seed morphology after planting on nutrient gel, far-red irradiation for 10 min and storage in the dark for indicated times (days). Seeds were planted, irradiated for 10 min, plates wrapped in foil, and stored at 25˚C. Images were taken using an environmental scanning electron microscope. Images are representative of plates from 3 separate experiments; scale bar = 100 µm. Note seed morphology is not obviously altered by the treatment/storage and there is no evidence of germination. (b) Seedling morphology after the indicated days of storage in the dark after far-red irradiation and then induction of germination and growth with white light. Seeds were planted, irradiated for 10 min, plates wrapped in foil, and stored at 25˚C for the indicated times and then unwrapped, placed in a growth chamber, and grown on for 11 days. 'No far-red' represents control samples where the seeds were planted on nutrient gel without far-red irradiation and then immediately moved to germinate under white lights. Representative images of n=3 separate experiments; scale bar = 1 cm. (c) Root length vs. time of growth post germination for plants held dormant by far-red irradiation for 0-21 days as noted. Data represent mean ± SEM, n=3. Values for samples held dormant for 0-21 days are not significantly different from each other at each time post germination (t-test, p>0.05).

CONCLUSIONS

In summary, the effects of the light irradiation system described are consistent with the findings of Nakashima et al. (2014) that a far-red treatment provides a viable approach to delaying seed germination for spaceflight experiments. The new far-red irradiation equipment design extends the throughput of this approach and with the implementation of a portable darkroom reduces the operational requirements for the lab to perform setup of the experiment. Although heat buildup is effectively mitigated by the water cooling system in this equipment, replacement with a Peltier cooler is an option to be explored, as it would obviate the need for a source of ice for chilled water, and so further reduce lab requirements for running the equipment to a single electrical outlet. As irradiation as short as 1 s can effectively induce dormancy in Arabidopsis, heat buildup may not be an issue when used as part of an intermittent, short duration irradiation protocol. However, when run continuously to process larger numbers of Petri plates, or when used with plants that may require longer far-red irradiation to impose seed dormancy, running the cooling system is likely to be an important element of an experiment's design.

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