Testing the Bio-compatibility of Aluminum PDFU BRIC Hardware

Eric R. Schultz¹, Agata K. Zupanska², Susan Manning-Roach³, Jose Camacho³, Howard Levine³, Anna-Lisa Paul¹,², and Robert J. Ferl¹,²,⁴

¹Plant Molecular and Cellular Biology Program, University of Florida, Gainesville, FL 32611; ²Horticultural Sciences, University of Florida, Gainesville, FL 32611; ³NASA – Kennedy Space Center, FL 32899; ⁴Interdisciplinary Center for Biotechnology Research, University of Florida, Gainesville, FL 32611

ABSTRACT

Biological research in an orbital environment necessitates the containment of the sample and its associated chemical fixatives. The Biological Research in Canisters (BRIC) hardware developed by Kennedy Space Center has been widely used in several configurations to support biological experiments on the Shuttle and the International Space Station (ISS). The current model of BRIC hardware contains six Petri Dish Fixation Units (PDFUs), each of which holds one Petri plate containing the specimen. This study compares traditional polycarbonate PDFUs to PDFUs primarily composed of aluminum with respect to their biocompatibility with Arabidopsis thaliana (Arabidopsis) growth and development. Seeds were planted on nutrient agar plates and inserted into either polycarbonate or aluminum PDFUs, which were then secured in the BRIC hardware. Plates were allowed to develop in the PDFUs in the dark for a period of 12 days, after which they were preserved by either RNAlater or glutaraldehyde, harvested, photographed, RNA-extracted, and prepared for gene expression analyses. Direct comparison of the etiolated Arabidopsis seedlings from the polycarbonate and aluminum PDFUs presented no discernible morphological differences, nor were there any significant differences between the expression levels of several target genes chosen for their sensitivity in reporting an aluminum stress response.

INTRODUCTION

NASA’s interest in the response of terrestrial biology to the spaceflight environment involved plants from the very beginning of space exploration, and has included research focused on the role of plants in bioregenerative life support as well as basic physiological responses (reviewed in Dutcher et al., 1994; Ferl et al., 2002; Halstead and Dutcher, 1987; Salisbury, 1990; Salisbury and Bugbee, 1988; Wolverton and Kiss, 2009). Conducting experiments in spaceflight conditions requires specialized hardware that can maintain plants in a habitat that supports growth and development with a minimal amount of stress, as well as isolate and protect both the samples being studied and components

Key words: Spaceflight Hardware; Space Biology; PDFU; BRIC; Chemical Fixation; Aluminum Toxicity; Plant Growth and Development; Microscopy; Indicator Genes

Correspondence to: Robert Ferl
University of Florida
Interdisciplinary Center for Biotechnology Research
Gainesville, FL, 32611-0690, USA
Telephone: (352) 273-8029
E-mail: robferl@ufl.edu

48 -- Gravitational and Space Biology Volume 26 (2) Oct 2012
vital to the operation of the spacecraft. One of the widely-used current designs originates with the Biological Research in Canisters (BRIC) project, designed for the containment of Petri plates and their chemical fixatives in a removable sub-compartment called a Petri Dish Fixation Unit or PDFU (Wells et al., 2001). Some versions of the BRIC hardware also have the ability to produce LED illumination from one side (Kern et al., 1999). This model of BRIC resembles a large, sealed, anodized-aluminum box to house PDFUs and environmental data loggers (Cox and Quincy, 2012). The current PDFUs used in space shuttle life sciences experiments are made of polycarbonate with aluminum lids, and have been used with several different plant systems (e.g., Kern et al., 2005; Millar et al., 2011; Paul et al., 2012). However, in pre-flight testing prior to use on the last Space Shuttle Mission (STS-135), the KSC Payload Development Team observed that in one newly fabricated batch of PDFUs there was an instance of occasional microscopic cracks within the PDFU polycarbonate bodies. These faults were attributable to either the material itself or stresses from hardware preparation procedures, including repeated autoclaving. This PDFU lot was removed from the flight hardware inventory due to the risk associated with fixative leakage on-orbit. Potential solutions involved refining the manufacturing and annealing processes used in PDFU construction. Another was to switch PDFU construction from polycarbonate to aluminum (MIL-A-8625, Type III, Class 2), which led to the design and fabrication of the aluminum PDFU bodies.

Anodization is an electro-chemical oxidation process utilizing weak acids and electrical current resulting in a strong coating of oxidized aluminum. The process increases the thickness of the naturally-formed and very stable Al2O3 layer. Anodized aluminum is known for its outstanding strength and durability, and is also non-conductive, highly resistant to scratching, corrosion, and salts, all of which demonstrate the desired properties for space vehicle hardware. The fixation port and vent holes of the new PDFUs were made of alodined aluminum using Alodine 600 solution (MIL-C-5541, Class 1A) as shown in Figure 1. The alodining process is the chemical application of a protective chromate conversion coating on aluminum; however the coating is not as durable as the anodized portions.

![Figure 1. Configuration of Aluminum PDFU hardware. Two views are shown: a top down view (left of both A and B), showing the Petri plate well and two screw points and a side view (right of both A and B), showing the chemical fixative well and two additional screw points. (A): PDFU Polycarbonate. The unit is constructed primarily of polycarbonate, with the only exceptions being the four screw points; (B) PDFU-Aluminum. The entire unit is comprised of solid aluminum, with the labeled regions indicating the different treatments used in the aluminum construction.](image-url)
Aluminum is the most abundant metal and the third most common element in the Earth's crust; it is a natural element in the plant growth environment. Aluminum exhibits complex chemistry. At mildly acidic or neutral pH, aluminum is primarily in the form of insoluble aluminosilicates or oxides (Al(OH)\(^2+\), Al(OH)\(^3+\), Al(OH)\(_3\) gibbsite or Al(OH)\(_4^-\) aluminate), all forms harmless to plants (Delhaize and Ryan, 1995; Kochian, 1995). However, aluminum cations are potentially toxic. Plants can absorb trivalent aluminum species (Al\(^3+\)), which occur naturally with acidification of soil resulting in the hydrolysis of aluminum (Stass et al., 2006). The primary site of aluminum accumulation and toxicity is in the root apex, including the root cap, meristem, and elongation zone, resulting in stunted root elongation (Delhaize and Ryan, 1995). The ratio of root length to hypocotyl length may easily be used as a sensitive physiological marker of aluminum toxicity. The radial swelling of roots is another aluminum-induced morphological aberration (Blancaflor et al., 1998; Zelinova et al., 2011). Once aluminum has been internalized into a cell, it can bind to cell walls and membrane lipids, and can also depolarize the plasma membrane and disrupt cytoskeletal organization (Blancaflor et al., 1998; Illes et al., 2006; Jones and Kochian, 1997). Aluminum disturbs calcium homeostasis and calcium-mediated signaling, which subsequently impacts multiple signal transduction pathways (Rengel and Elliott, 1992). Aluminum represses mitochondrial activities, respiration inhibition, and ATP depletion, which triggers the production of reactive oxygen species (ROS) (Yamamoto et al., 2002).

There are few known aluminum resistance mechanisms. One includes the external detoxification of aluminum by releasing–organic compounds (such as citrate, malate, or oxalate) that bind to harmful aluminum cations (Ma, 2007; Ma and Furukawa, 2003). Another is an internal mechanism, which involves root cells producing the defense-related cell wall polysaccharide callose in response to aluminum exposure (Jones et al., 2006; Sivaguru et al., 2000). Callose brings rigidity to the cell wall by cementing cell walls together, therefore closing the plasmodesmata and sealing the passage of solutes.

It is well established that polycarbonate PDFUs provide a benign habitat for Arabidopsis growth; however, aluminum PDFUs have yet to be tested for biocompatibility. We hypothesized that the aluminum housing materials would be equally compatible with Arabidopsis plants as the polycarbonate counterparts; since the biology is contained within Petri plates that are inserted into individual PDFUs, there is no direct contact of the biology with the wall materials. Further, the alodined aluminum compartment, which could potentially release chromium ions, is spatially distant from the plate and cannot contribute to the microenvironment defined by the PDFU (Figure 1). To test the hypothesis, we assessed whether predominantly aluminum PDFUs had any impact on the growth, development, or health (as monitored by expression of selected stress-induced genes) of Arabidopsis compared to Arabidopsis grown in polycarbonate PDFU controls. We observed the morphology of the seedlings, primarily root morphology with microscopy, coupled with molecular analysis to assess whether housing Petri plates in aluminum PDFUs causes any deviations from polycarbonate-grown samples. In addition, we also examined the extreme scenario of direct exposure of Arabidopsis seedlings to uncoated or alodined aluminum punch-out blocks (“coupons”) in growth media.

**MATERIALS AND METHODS**

**Experimental Design and Plant Growth**

Arabidopsis seeds (30-40) were planted on 60 mm Petri plates filled with 7 mL standard nutrient media as seen in Figure 2 for a total of 21 plates. Once planted, six plates were transferred to Polycarbonate PDFUs (PDFU-Polycarb control) and six others were transferred to aluminum PDFUs. The remaining nine plates were divided into three bench top trials—three in a light-tight box (Bench Top control), three with an added aluminum coupon (Aluminum 7075) placed in the agar, and three with a similar aluminum coupon treated with an alodine solution. Aluminum coupons were dipped in alodine/chromate solution, allowed to dry, washed three times in sterile distilled water, and then washed in 70% ethanol. These trials were labeled BT, BT Al-Coupon, and BT Al-Alodine,
respectively, and did not take place in BRIC hardware. Plates were housed vertically in all five configurations and held at ambient room temperatures (21–23°C) in the dark. The organization of the PDFUs is provided in Figure 3. The PDFU ID numbers are shown on each unit, along with their corresponding letter designation: Polycarbonate 1004, 1020, 1021, 1026, 1028, 1030 (A1–A6, respectively) and aluminum X001, X002, X003, X004, X005, X006 (B1–B6, respectively). Three of the PDFUs in each set were fixed with RNAlater, and three with glutaraldehyde (Figures 3-5). Plants were grown for 12 days before fixation in RNAlater or glutaraldehyde and photographed individually following their removal from their respective PDFUs. Additional details for PDFU harvest and sample processing can be found in Paul et al. (2012).

Figure 2. Seeded plate (A, B) and insertion into PDFUs (C, D). Arabidopsis seeds were planted on nutrient agar plates and inserted into the BRIC hardware containing either polycarbonate or aluminum PDFUs. Plating was performed under sterile conditions in a laminar flow hood.

**Microscopy**

Individual plants were harvested from plates following extraction from PDFUs. Micrographs were taken on a dissection microscope with plants being transferred to 5 mL screw-top tubes of their respective fixatives for storage and further analysis. RNAlater samples were saved for RNA expression analysis, while glutaraldehyde-fixed samples received further visual inspection. Six to seven seedlings from each Petri plate were photographed in detail (therefore n=20 per treatment) and subsequent micrographs were processed in ImageJ (Schneider et al., 2012) to measure the length of the hypocotyls and the length of the primary root. Cellular morphology was analyzed using a compound light microscope. The root/hypocotyl ratio was calculated, as well as a mean and standard deviation for each treatment. These data were then used in a two-sample t-test for comparing two means, using the following
Figure 3. Complete set of assembled PDFUs. Individual PDFUs were labeled with a single letter and number combination for identification. The PDFU ID numbers are shown on each unit, along with their corresponding letter designation: Polycarbonate 1004, 1020, 1021, 1026, 1028, 1030 (A1–A6, respectively) and aluminum X001, X002, X003, X004, X005, X006 (B1–B6, respectively). PDFUs A1-A3 and B1-B3 were fixed in glutaraldehyde, while A4-A6 and B4-B6 were fixed in RNAlater.

Figure 4. Petri plates from PDFUs fixed with RNAlater. Each 60 mm plate is shown photographed against a black cloth background to highlight the general growth habit of the etiolated seedlings. Top Row: PDFU-Polycarb (A) 1004; (B) 1020; (C) 1021; Bottom row: PDFU-Aluminum (D) X001; (E) X002; (F) X003.
equation, where $\bar{x}_C$ is the mean of the control data set, $\bar{x}_E$ is the mean of the experimental data set, $s$ is the standard deviation, and $n$ is the number of measurements in each data set.

$$t = \frac{\bar{x}_C - \bar{x}_E}{\sqrt{\frac{s^2}{n_C} + \frac{s^2}{n_E}}}$$

Bench top control was used for comparisons with the other two bench top experiments (BT vs. BT Al-Coupon and BT vs. BT Al-Alodine), while the polycarbonate PDFU data were used exclusively for comparison with the aluminum PDFU growing condition.

**RNA Extraction**

Total RNA was extracted using Qiashredder and RNAeasy™ kits from QIAGEN (QIAGEN Sciences, MD, USA) according to the manufacturer’s instructions. Residual DNA was removed by performing an on-column digestion using an RNase Free DNase (QIAGEN GmbH, Hilden, Germany). Integrity of the RNA was evaluated using the Nanovue Spectrophotometer (GE Healthcare) and absorbance measurements at A260, with a test ratio of A260/A280 for purity.

**Conducting Real Time PCR (RT-qPCR)**

The transcript levels of the three aluminum-response target genes were evaluated by quantitative RT-PCR. Eight-hundred fifty (850) ng of total RNA were reverse transcribed into cDNA using High Capacity RNA to cDNA Master Mix (Applied Biosystems, Foster City, CA, USA). One-tenth (1/10) of total cDNA was used as a template for a single RT-qPCR run. RT-qPCR was carried out using TaqMan technology on the ABI 7500 Fast instrument (Applied Biosystems, Foster City, CA, USA). The TaqMan™ Fast Advanced Master Mix (Applied Biosystems, Foster City, CA, USA) reagent was used for the duplex RT-qPCR reaction with 6FAM and VIC-dye labeled, TAMRA-quenched probes. Primers and probes were designed with Primer Express software and supplied by Applied Biosystems. The primers/probes sequences shown as 5’→3’ may be seen in Table 1.

**Table 1. Primer/Probe sequences used for RT-qPCR.** Primers and probes (shown 5’→3’) were designed with Primer Express software and supplied by Applied Biosystems.

<table>
<thead>
<tr>
<th>Target</th>
<th>Gene</th>
<th>Forward</th>
<th>Reverse</th>
<th>Probe</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ubq11</td>
<td>At4g05050</td>
<td>AACTTGAGGACGCCAGAACTTT</td>
<td>GTGATGGTCTTTCCCGTCTAAA</td>
<td>VIC-CAGAAGGAGTCTACGC TTCATTGTTGCTTGCT-TAMRA</td>
</tr>
<tr>
<td>DDF1</td>
<td>At1g12610</td>
<td>CAGAGAACCGACGCACCAACACCA</td>
<td>GCACGCGCTGCCAT ATCT</td>
<td>6FAM-CCGCAATTTGCGCTCAGG ACTTATCC-TAMRA</td>
</tr>
<tr>
<td>TCH4</td>
<td>At5g57560</td>
<td>TGGAACCCCAAAAGAATCATTTT</td>
<td>GTGCCTAGAGACTCC ATGTTCCTG</td>
<td>6FAM-ACCGTCGATGGAAACTC CGATCAGAGA-TAMRA</td>
</tr>
<tr>
<td>AtMATE</td>
<td>At1g51340</td>
<td>CAGGTGTGGTTAGCTACTTTTACTTTT</td>
<td>AACGCCTCGCTAGT ATTGC</td>
<td>6FAM-CAGACGGGTACGCC CTGCTTGCC-TAMRA</td>
</tr>
</tbody>
</table>
RT-qPCR was performed as reported previously (Paul et al., 2012). Briefly, in all reactions the Ubq11 served as an internal control. Each duplex PCR mixture contained 900 nM target gene-specific forward and reverse primers each, 150 nM Ubq11 forward and reverse primers each, 250 nM 6FAM labeled target gene-specific probe, and 250 nM VIC-labeled Ubq11 probe. The thermal cycling program consisted of 20 s at 95°C, followed by 40 cycles of 3 s at 95°C, and 30 s at 59°C. Reactions were quantified by selecting the amplification cycle when the PCR product was first detected (threshold cycle, Ct). Primers and probe sets were first subjected to validation experiments to test the efficiency of the target and reference amplifications. The Ct values for respective number of biological replicas of each experimental group (treated, control) were analyzed using 7500 Software v2.0.5 along with Microsoft Excel and the comparative CT(ΔΔCT) method. The ΔCt was calculated as the difference between the threshold cycle value of a target gene and that of Ubq11 (endogenous control) in the same sample, while ΔΔCt as the difference between the ΔCt value of a treated sample and that of the control (calibrator). The fold difference of the target genes expression in treated samples relative to control samples (calibrator) was calculated as $2^{-\Delta\Delta Ct}$ and then log2-transformed.

**RESULTS**

**Growth Habit Overview**

There were no observable differences between seedlings grown in the polycarbonate PDFUs and the aluminum PDFUs. There was also no difference between seedlings preserved with RNAlater or glutaraldehyde in either PDFU configuration (Figures 4 and 5). The initial survey photographs of the plates as they were calculated as the difference between the threshold cycle value of a target gene and that of Ubq11 (endogenous control) in the same sample, while ΔΔCt as the difference between the ΔCt value of a treated sample and that of the control (calibrator). The fold difference of the target genes expression in treated samples relative to control samples (calibrator) was calculated as $2^{-\Delta\Delta Ct}$ and then log2-transformed.

**RESULTS**

**Growth Habit Overview**

There were no observable differences between seedlings grown in the polycarbonate PDFUs and the aluminum PDFUs. There was also no difference between seedlings preserved with RNAlater or glutaraldehyde in either PDFU configuration (Figures 4 and 5). The initial survey photographs of the plates as they were

![Figure 5. Petri plates from PDFUs fixed with glutaraldehyde. Each 60 mm plate is shown photographed against a black cloth background to highlight the general growth habit of the etiolated seedlings. Top Row: PDFU-Polycarb (A) 1026; (B) 1028; (C) 1030; Bottom row: PDFU-Aluminum (D) X004; (E) X005; (F) X006.](image-url)
removed from the PDFUs at the end of the experiment showed no visible differences between the two different PDFU materials. Closer inspection through individual plate photographs confirms a lack of differences in growth habit between those plants grown in polycarbonate PDFUs and those grown in aluminum PDFUs (Figures 4 and 5).

In the bench top trials seen in Figure 6, the uncoated aluminum coupons appeared to have little impact on seedling growth morphology (Figure 6, middle row); however, seedlings in direct contact with the alodine-coated coupons appeared to have significantly stunted root growth (Figure 6, bottom row).

**Evaluations of Seedling Morphology with Microscopy**

There were no statistically significant differences in root length or in hypocotyl length between seedlings grown in the polycarbonate PDFUs and the aluminum PDFUs. However, a significant impact (p < 0.001) on growth exists in seedlings in immediate contact with uncoated and minimally washed alodine-treated aluminum.

The morphometric analyses of the polycarbonate and aluminum PDFU grown plates were based on the ratios of the hypocotyl length to primary root length in representative plants. These analyses were also conducted on the Bench-Top (not hosed in PDFUs) trials in the presence of either plain aluminum coupons or alodine coated aluminum coupons and were compared to the untreated Bench-Top seedlings. Representative photographs from each treatment are provided in Figure 7.

Visual inspection suggested that there was little or no difference between the PDFU conditions (Figure 7A and 7B), a conclusion supported by the similar cellular morphology of polycarbonate PDFU-grown and Aluminum PDFU-grown roots seen in Figure 8. In Bench-Top trials visual inspection indicated that there was little or no difference in the morphology between the control plants and those grown in contact with bare, untreated aluminum (Figure 7C and 7D). However, plants grown in contact with alodine-coated aluminum displayed significantly stunted root growth (Figure 7E).

Quantitative analyses of root and hypocotyl length were conducted with six to seven seedlings from each Petri plate in each treatment (n=20 for each treatment). An average ratio was calculated for each condition along with its standard deviation and plotted in Figure 9. Error bars indicate one standard deviation away from the mean. It is important to note that the Bench-Top trials (dark red bars) cannot be compared directly to the PDFU tests (dark blue bars) due to a slight difference in lighting exposure which may impact the root/hypocotyl ratios. Using the equation described in the methods section, these data show that no significant difference exists between the polycarbonate PDFU seedlings and the aluminum PDFU seedlings (p > 0.30). Growing plants in direct contact with aluminum coupons (untreated and alodine treated) inhibited root growth and significantly altered the root/hypocotyl ratios (p < 0.001 for both conditions).

**RNA Integration and Toxic Stress-Induced Transcript Abundance**

There was no discernible difference in the quality and purity of the RNA extracted from seedlings under any of the treatment conditions. The RT-qPCR results were calculated in two ways: all PDFUs and coupon treatments relative to the Bench-Top untreated control (BT), shown in Figure 10A, and PDFU-Aluminum relative to PDFU-Polycarb (Figure 10B). None of the three targets (TCH4, AtMATE, and DDF1) were upregulated in either of the PDFU configurations (PDFU-Polycarb vs. PDFU-Aluminum) relative to BT control (Figure 10A). The fold change calculated as log2 function was never higher than 0.5 (TCH4, DDF1) and not lower than -1 (AtMATE). For all three targets in the PDFUs samples, the values fell within margin of error; therefore, not only were changes in expression minor, but certainly not statistically significant. However all three targets showed enhanced expression in both BT Al-Coupon and BT Al-Alodine samples relative to the untreated control, BT. In BT Al-Alodine samples, the target genes overexpression was significant from 4.75 times more of TCH4 (log2=2.25), to 11.3 times more of DDF1 (log2=3.5), and the highest 16 times more of AtMATE (log2=4) comparing to BT. Still, the highest upregulation of all three transcripts was
Figure 6. Additional Bench-Top (BT) tests outside of the PDFU hardware. These treatments should primarily be compared amongst themselves, not to plants grown in the BRIC hardware. Top Row: BT controls (BT) (A) B001; (B) B002; (C) B003; Middle row: Grown with aluminum coupon inserted in media (BT Al-Coupon) (D) B004; (E) B005; (F) B006; Bottom row: Grown with alodine-coated aluminum coupon inserted in media (BT Al-Alodine) (G) B007; (H) B008; (I) B009.
Figure 7. Microscopy of seedling morphology from PDFUs and additional Bench-Top (BT) tests. Top Row: Representative samples from (A) PDFU Polycarbonate; (B) PDFU-Aluminum; Bottom Row - (C) BT control (BT); (D) BT Al-Coupon; (E) BT Al-Alodine. The shortened roots of the alodine-associated samples are indicated with yellow arrows.
Figure 8. Light microscopy of glutaraldehyde-fixed seedling morphology from PDFUs. Representative samples from (A) PDFU-Aluminum; (B) PDFU-Polycarbonate. Scale bars: 200 μm.

Figure 9. Root / Hypocotyl ratios among treatments. Dark blue bars represent PDFU-Polycarb vs. PDFU-Aluminum; Dark red bars represent BT Bench Top control vs. BT Al-Coupon and BT Al-Alodine. Error bars indicate one standard deviation away from the mean, n=21 for each treatment. ns = no significant change; * = p < 0.001; **= p < 0.000001.
Figure 10. Transcript levels of target genes in the different treatments. The AtMATE, DDF1, TCH4 transcripts in seedlings fixed with RNAlater were quantified with RT-qPCR. The x-axis represents the fold change of gene expression calculated as a log2 function. (A) - Transcript levels relative to Bench-Top control (BT). (B) - Transcript levels in PDFU-Aluminum relative to PDFU-Polycarb. The RT-qPCR data shows the mean for three biological replicas for each gene. Error bars represent log function of 2-ddCT SEM.

observed in BT Al-Coupon samples, with 11.3 times more of both TCH4 and AtMATE \((\log_2=3.5)\) and close to 35 times more of DDF1 \((\log_2=5.15)\).

The second approach to calculate RT-qPCR relative expression was applied, in which the transcripts abundance in PDFU-Aluminum samples was calculated relative to PDFU-Polycarb. This calculation would allow the direct comparison of how PDFU material impacted the changes in gene expression. None of the target genes showed significant change of expression when comparing PDFU-Aluminum to PDFU-Polycarb (Figure 10B). Fold change \((\log_2)\) of -0.25 (DDF1) or -0.3 (AtMATE) was not statistically significant.
DISCUSSION

Several metrics were used to evaluate the plant-biocompatibility of BRIC PDFUs constructed from aluminum: growth habit, organ morphology, and selected gene transcript abundance. Growth habit was both visually inspected for obvious anomalies and quantified with respect to the ratio of hypocotyl to root length. We found no statistical difference in the root to hypocotyl length ratios of plants grown in the polycarbonate PDFUs compared to those grown in the aluminum PDFUs. The identical growth habits can likely be attributed to their growth in identical media and Petri plates, and as such, never coming in contact with the PDFU itself. The root to hypocotyl ratio confirmed the hypothesis formed during basic observation by indicating an insignificant difference between the two treatments. Further, there were no visible morphological aberrations in root or shoot structures, such as swelling from callose formation, or deformed root hairs, that would suggest a difference in growth and condition on plates housed in the two PDFU compositions.

Altered root morphology did appear during the Bench-Top trials, but only when in close proximity with the aluminum coupon or aluminum-alodine coupon, with the alodine coupon severely stunting root growth. This increase in stressed morphology is likely due to the additive effect of chromium (VI) being released onto the growth medium and causing its own stress in addition to the aluminum (Ozdener et al., 2011; Rodriguez et al., 2011).

Patterns of gene expression can reflect a plant’s metabolic response to its environment. To understand if Arabidopsis seedlings housed in aluminum PDFUs mounted any molecular response different from samples held in polycarbonate PDFUs, we examined transcript abundance of three target genes as representatives of potential stress response genes. Target gene selections were also founded on the data collected from our study of the BRIC-16 experiment performed on the STS-131 Discovery Space Shuttle mission, which included etiolated Arabidopsis seedlings housed in polycarbonate PDFUs in BRICs (Paul et al., 2012). The first gene target, DDF1 (Dwarf and Delayed Flowering 1), constitutes a general stress response gene that would likely remain unaffected in case the PDFU aluminum material turned toxic. The DDF1 encodes a member of the DREB subfamily A-1 of ERF/AP2 transcription factor family and belongs to the larger family of transcription factors participating in response to multiple environmental stresses, including cold, high salinity, and drought. In our BRIC-16 experiment, DDF1 was unaffected in polycarbonate PDFUs on the ground (Ground Control samples) and was also unaffected in Spaceflight samples (Experiment E-MTAB-1009 in ArrayExpress database) (Paul et al., 2012). The second gene, TCH4 (xyloglucan endotransglucosylase/hydrolase protein 22), is another general stress response gene that was also reported to be overexpressed in response to aluminum toxicity (Experiment E-GEOE-7334 in ArrayExpress database) (Goodwin and Sutter, 2009; Lee et al., 2005). The induction of aluminum toxicity occurred when Arabidopsis seedlings were removed from agar plates to directly contact aluminum by submersion into a solution containing 50 µM aluminum for 16 hours. TCH4 encodes a cell wall-modifying enzyme believed to play a role in general detoxification. In our BRIC-16 experiment, TCH4 expression level in Ground Control samples held in polycarbonate PDFU was slightly higher than in Spaceflight samples. Therefore, TCH4 could be a part of the molecular response of seedlings to the spaceflight environment rather than to PDFU materials (Experiment E-MTAB-1009 in ArrayExpress database) (Paul et al., 2012). The third target gene, AtMATE, is an aluminum toxicity-specific response gene. This gene encodes a plasma membrane-localized citrate transporter that belongs to the multi-drug and toxic compound extrusion (MATE) family (an efflux family), which exhibits antiporter and drug transmembrane transporter activity (Magalhaes et al., 2007). AtMATE is expressed primarily in roots and is induced specifically by aluminum toxicity as a part of the important resistance mechanism, although its expression has been primarily studied in aluminum solutions of 50 µM (16 hrs) or 25 µM AlCl3 (6 or 48 hrs) (Experiment E-GEOE-7334 in ArrayExpress database from Griesel et al. and Supplemental Table 1 in Kumari et al., respectively; Grisel et al., 2010; Kumari et al., 2008). Also AtMATE
functions independently from AtALMT1, an aluminum activated malate transporter to confer aluminum tolerance and is consequently a very strong candidate for a specific aluminum-responsive gene (Liu et al., 2009). In our BRIC-16 experiment, AtMATE was downregulated in Spaceflight seedlings held in the PDFU polycarbonate environment (Experiment E-MTAB-1009 in ArrayExpress database) (Paul et al., 2012).

None of the target genes were differentially expressed in a direct comparison between the polycarbonate PDFU-grown plants and the aluminum PDFU-grown plants. Therefore, we conclude that growing in the aluminum PDFU was not perceived as a stress by seedlings, which in turn suggests that the aluminum composition of the PDFU housing was not toxic as it failed to trigger aluminum toxicity responsive genes.

However, all three target genes showed enhanced-expression when growing media which had direct contact with aluminum coupons. Aluminum and alodine-aluminum coupons both induced strong upregulation of DDF1, TCH4, and AtMATE. When combined with the morphology data, we conclude that the direct contact of aluminum coupons with the growth media elicits the signature aluminum stress response, implying that prolonged, direct contact with aluminum could become toxic. However, it is important to reiterate that in the normal operational configuration of the BRIC PDFU the plants and their growth medium do not come into direct contact with an aluminum surface.

CONCLUSIONS

We have demonstrated that changing the material utilized to build the PDFU had no impact on the condition of etiolated Arabidopsis seedlings as measured by growth, morphology, and selected gene transcript abundance. Therefore, we conclude that there are no plant-biocompatibility issues associated with the aluminum PDFUs. Although we did demonstrate that the direct contact with bare aluminum- or alodined aluminum- coupons elicits changes in morphology and in target genes expression, it is important to note that such a situation is outside any foreseen scenario for any biological experiment conducted in the BRIC hardware. In an actual spaceflight experiment in the BRIC PDFU hardware, neither the Arabidopsis plants nor the media on which they are grown would come in direct contact with the housing materials of a PDFU, as the plants would always be contained within a Petri plate inserted into the PDFU chamber.

REFERENCES


