Patterns of Arabidopsis gene expression in the face of hypobaric stress

Anna-Lisa Paul1†, Mingqi Zhou1†, Jordan B. Callaham1, Matthew Reyes2, Michael Stasiak3, Alberto Riva4, Agata K. Zupanska1, Mike A. Dixon3 and Robert J. Ferl1,4*

1 Program in Plant Molecular and Cellular Biology, Department of Horticultural Sciences, University of Florida, Gainesville, FL 32611, USA
2 Exploration Solutions, Inc., Moffett Field, CA 94035, USA.
3 University of Guelph, Guelph, N1G 2W1 ON, Canada.
4 Interdisciplinary Centre for Biotechnology, University of Florida, Gainesville, FL 32610, USA.

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†These authors share first authorship.

Abstract. Extreme hypobaria is a novel abiotic stress that is outside the evolutionary experience of terrestrial plants. In natural environments, the practical limit of atmospheric pressure experienced by higher plants is about 50 kPa or about 0.5 atmospheres; a limit that is primarily imposed by the combined stresses inherent to high altitude conditions of terrestrial mountains. However, in highly controlled chambers, and within projected extra-terrestrial greenhouses, the atmospheric pressure component can be isolated from the associated high altitude stresses such as temperature, desiccation and even hypoxia. Such chambers allow the exploration of hypobaria as a single variable that can be carried to extremes beyond what is possible in terrestrial biomes. Here, we examine the organ-specific progression of transcriptional strategies for the physiological adaptation to various degrees of hypobaric stress, as well as the response to severe hypobaria over time. An abrupt transition from a near-sea level pressure of 97 kPa to a mere 5 kPa is accompanied by the differential expression of hundreds of genes, primarily those associated with drought, hypoxia and cell wall metabolism. However, pressure transitions between these two extremes reveal that plants respond with complex, organ-specific transcriptomic responses, which also vary over time. These responses are not linear; neither with respect to the gradient of hypobaria severity from 75, 50, 25 to 10 kPa, nor with the duration of exposure of up to 3 days at 10 kPa. In the first few hours of hypobaria, plants engage changes in basic metabolism and hormonally mediated growth and development. After 12 or more hours of hypobaria, the gene expression patterns are more indicative of hypoxia and drought environmental responses. The hypobaria transcription patterns were highly organ specific, and roots appeared to be more sensitive to hypobaria than shoots in that the number of differentially expressed genes was higher in roots than in shoots. The patterns of gene expression among organs, across a gradient of atmospheric pressures and over time suggest that plants adapt to the novel stress of pure hypobaria by using recognizable metabolisms to meet appropriately interpreted hypoxia stresses, while engaging drought responses that are seemingly inappropriate to the wet and humid environment of the chambers.

Keywords: Abiotic stress; Arabidopsis; hypobaria; hypoxia; Mars greenhouse; reduced atmospheric pressure.

* Corresponding author’s e-mail address: robferl@ufl.edu

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Introduction

Current biogeographical boundaries are the result of evolutionary selection for capacity to thrive in certain environments. Excursion beyond those boundaries imposes stress, which is met by further engagement of pathways that evolved to adapt to the environment in which the stress is grounded. We are interested in probing the stress response contingencies that exist within plants by using novel abiotic stresses that have not been experienced over evolutionary time.

Hypobaria, low atmospheric pressure, is one such novel stress. In exploration of environments beyond Earth’s surface, altered atmospheric pressure can present both a crucial challenge, and an operational trade space for life support (Wieland 1998). Environmental conditions in spaceflight vehicles have traditionally been hypobaric to reduce the masses of structural materials to load more supplies and increase mission lengths. Concepts for orbital and planetary greenhouses often include reduced-pressure atmospheres to decrease the costs of energy and consumable components (Martin and McCormick 1992), and the living plants within those hypobaric habitats must be able to thrive to successfully maintain the life support system (Salisbury 1999). Thus growing plants in low atmospheric pressure is both a necessary consideration for human space exploration and an opportunity for the study of physiological adaptation contingencies.

The pressure at which plants can grow naturally on earth is limited by physical altitude (e.g. 35 kPa—kilopascal—at the top of Mount Everest) and the temperature, hypoxia and water stress that accompany extreme altitudes. A combination of environmental factors makes the natural terrestrial limit for higher plants about 50 kPa, half of the 101 kPa that defines the barometric pressure at sea level (Paul et al. 2004; Paul and Ferl 2006). Thus, an atmospheric pressure below 50 kPa represents an environment to which no higher plant has ever had to adapt. And yet, in artificial environments, plants can manage quite well at pressures as low as 10 kPa (Paul et al. 2004) and there have been studies showing that as long as the hypoxic component of hypobaria is mitigated, seeds can germinate and then grow at 25 kPa for over a month (He et al. 2007; He et al. 2009; He and Davies 2012). This adaptability has long been the foundation of the concept of utilizing reduced pressure greenhouses to support human crews on the moon or Mars (Corey et al. 1996; Clawson et al. 1999; Fowler et al. 2000; Goto et al. 2002; Richards et al. 2006; Paul and Ferl 2006), and yet current understanding of the molecular basis for plant adaptability at low pressures is limited.

In the laboratory, atmospheric pressure can be isolated from other high altitude stresses and hypobaria can be explored as a specific probe for examining plant environmental response contingencies. When faced with a novel stress, plants can respond in several conceivable ways. They can do nothing—if there is no receptor for the stress, nothing can be perceived to generate a response. They can respond appropriately—if there is a recognizable component of the stress (e.g. reduced oxygen availability) that triggers an adaptive response. Or they can mount an inappropriate response—if the novel stress initiates a response that does not contribute to survival. The latter two courses appear to manifest in the response of Arabidopsis thaliana (Arabidopsis) to a severe hypobaric environment. Arabidopsis responded to a 24 h hypobaric exposure (from 101 to 10 kPa), with a unique utilization of the genome to cope with a novel situation, a response which was not equal to a comparable hypoxic environment at 101 kPa. 24 h at 10 kPa resulted in the differential expression of over 200 genes in Arabidopsis leaves, and central among these genes were those of ABA and drought metabolisms. However, the induction of many of the genes in this set reflects an inappropriate response in that the plants were not actually water stressed, yet responded as if they were by engaging drought response metabolisms (Paul et al. 2004).

Given the seemingly inappropriate response of 24 h direct exposure to 10 kPa, we wished to examine the onset and development of the response over both time and severity of hypobaria. We also wished to examine the organ specificity of the response to better understand the physiological basis of the response. We present here a compilation and comparison of the changes in gene expression patterns that comprise the response of Arabidopsis shoots and roots to hypobaric conditions ranging from mild (75 kPa), moderate (50 kPa) and severe (25 kPa) to extreme (10 and 5 kPa). We also explore the effect of time, evaluating the gene expression patterns as plants acclimate to extreme hypobaria (10 kPa) after 1, 3, 6, 12, 48 and 72 h.

Methods

Reduced atmospheric pressure chambers and facilities

The Low Pressure Growth Chambers (LPGC) used in this experiment were part of the Controlled Environment Systems Research Facility (CESRF) at University of Guelph, Ontario, Canada (Fig. 1A). The LPGC were programmed with variable conditions depending on each sub-experiment treatment. Each chamber was monitored at 5 min intervals and controlled for temperature, air pressure and gas composition. Relative humidity and vapour pressure density were also monitored. Due to the
location of CESRF in Guelph, Ontario at 1100 feet above sea level, the control pressure for all experimental conditions was set at 97 kPa.

Plant preparations

Arabidopsis thaliana (ecotype Wassilewskija) were used throughout. Seeds were surfaced sterilized with a 5-min rinse of 70% Ethanol, a 50% bleach (v/v) and Tween 20 soak for 10 min, followed by multiple rinses of sterile water. Seeds were then planted on vertically orientated Petri plates (10 cm²) containing 0.5× MS media (Murashige and Skoog 1962), 0.45% Phytagel (w/v) and 2.5 ppm benomyl. Plates were sealed with Micropore (3 M) tape to allow air exchange (Paul et al. 2001).

Prior to atmospheric treatments, plants were grown in an ambient pressure growth chamber at CESRF. Growth chamber conditions were: 24-h light, 22–25 °C, humidity of 95% or above maintained inside the Petri plates and ambient atmosphere of 97 kPa (Guelph, ON ambient).

Experiment 1—variations on a pressure

A set of 10-day-old plants grown vertically on plates were transferred to the LPGCs and exposed to six different atmospheric pressures for a period of 24 h; 97, 75, 50, 25, 10 and 5 kPa. An example of how the plates were oriented and contained within the LP chambers is shown in Fig. 1B and C. In all treatments, the carbon dioxide was kept consistent at a partial pressure of 0.05 kPa. In each treatment, oxygen was kept at 21% (v/v) of the total chamber pressure with Nitrogen as a balance of remaining gas. Each treatment of atmospheric pressures and gases was replicated with three separate, concurrently run LPGCs. Each chamber in the LPGCs contained 10 plates, with ~12 plants on each plate.

Experiment 2—a 10 kPa time course

A set of 10-day-old plants grown vertically on plates were exposed to 10 and 97 kPa in LPGCs and sampled at six different time points. The 97 kPa atmosphere was composed of partial pressures of 21 kPa oxygen, 0.05 kPa carbon dioxide and a balance of nitrogen. The 10 kPa samples were composed of a partial pressure of 2.1 kPa oxygen, 0.05 kPa carbon dioxide and a balance of nitrogen. The samples were harvested at: 1, 3, 6, 12, 48 and 72 h. Each treatment of atmospheric pressures and times were replicated with three separate, concurrently run LPGCs. Each chamber in the LPGCs contained 10 plates of ~12 plants each.

Harvest, extraction and analysis

At the end of the treatment plates were removed from the chambers, opened and plants were harvested using forceps directly to a fixative RNAlater (Ambion). Each plate of ~12 plants was harvested to a separate tube, for a total of 10 tubes per treatment. To allow for later organ specific gene expression analysis, some samples were...
separated into aerial parts (shoots) and roots before being removed from the plate, and as quickly as possible submerged in RNAlater to avoid wounding responses. Samples were immediately stored according to manufacturer recommendations, with long term storage at \(-20^\circ\text{C}\) until extraction. A random selection of three tubes was used from each sample group for RNA extraction. Total RNA was extracted from the samples using Qiashredder columns in the Qiagen RNAeasy kit and removed residual DNA using RNase-free Dnase. RNA concentration was determined on a BioSpectrometer (Eppendorf) and sample quality was assessed using the Agilent 2100 Bioanalyzer (Agilent Technologies, Inc.). Samples were then subjected to microarray analysis.

**Microarray assay**

The 100 ng of total RNA from each sample was reverse transcribed into double-stranded cDNA, from which biotin-labelled cRNA was generated using the 3’ IVT plus Kit (Affymetrix). The cRNA was purified using magnetic beads and was fragmented. Following fragmentation, cRNA products (12.5 µg) were hybridized with rotation to the Affymetrix GeneChip® Arabidopsis ATH1 Genome Arrays for 16 h at 45 °C. Arrays were washed on a Fluidics Station 450 (Affymetrix) using the Hybridization Wash and Stain Kit (Affymetrix) and the Washing Procedure FS450_0004. Fluorescent signals were measured with an Agilent 2100 Bioanalyzer (Agilent Technologies, Inc.). The arrays were normalized using the RMA method, and differential analysis was performed using the 'Limma' R package (Ritchie et al. 2015). Data quality was assessed using arrayQualityMetrics package and various QC charts (Density & Intensity plot, NUSE, RLE and RNA Degradation Plot). For each replicate array, each probe-set signal value from treated samples was compared to the probe-set signal value of control samples to give gene expression ratios. Differentially expressed genes were identified using Limma package with a Benjamini and Hochberg false discovery rate multiple testing correction. Genes were considered as differentially expressed with stringent criteria at \(P < 0.01\), Log2 (fold change) > 1. The \(P\) values for each treatment comparison are included in the Supporting Information Tables S1 and S2.

The differentially expressed genes in the two tissues were clustered with the GENE-E program (http://www.broadinstitute.org/cancer/software/GENE-E/index.html) using Kendall tau distance (Eisen et al. 1998; Saldanha 2004; Cai et al. 2012). Gene ontology analysis of biological process was performed using AgriGO (http://bioinfo.cau.edu.cn/agriGO/index.php) (Du et al. 2010) and GO terms of biological process with \(P < 0.01\) were listed in Supporting Information Tables S1 and S2. The pathway enrichment analysis was performed with DAVID6.8 (https://david.ncifcrf.gov/) using Kyoto Encyclopaedia of Genes and Genomes (KEGG) database (http://www.genome.jp/sigtool/map_pathway1.html (13 July 2017)).

**Table 1.** The number of successful arrays for each treatment.

| Experiment 1: Variations on a Pressure | Experiment 2: A 10 kPa Time Course |
|---------------------------------------|-----------------------------------|
| kPa 97                                 | 10 kPa Time Course                |
| Time (h) 24                           |                                   |
| Roots 3                                | 10 10 10 10                        |
| Shoots 3                              | 10 10 10 10                        |

**Quantitative RT-PCR**

Quantitative RT-PCR was used to quantify the expression levels of genes selected from the microarrays data. Quantitative RT-PCR reactions were conducted in triplicate with the same RNAs as were used for the microarray analyses, and the reactions were analysed with the Applied Biosystems Prism 7700 Sequence Detection System (Paul et al. 2004). The fluorescently tagged probes and paired primers flanking a 60–100 bp section of the gene of interest are listed in Table 2. The gene
expression level was normalized to a standard curve. UBQ11 (AT4G05050) was used as the internal control.

Results
Utilization of low-pressure environments to challenge Arabidopsis acclimation
The low pressure growth chambers (LPGC) at the University of Guelph's Controlled Environment Systems Research Facility (CESRF) were configured to provide several hypobaric atmosphere environments, including an ambient pressure (97 kPa) control (Fig. 1A). In Experiment 1, the plants were grown for 24 h in LPGC at atmospheric pressures of 97, 75, 50, 25, 10 and 5 kPa. In Experiment 2, the seedlings were grown in either a 10 or a 97 kPa environment to establish a time course comprised of 1, 3, 6, 12, 48 and 72 h. The 24 h time point was represented in Experiment 1, and so was not repeated in the Experiment 2 time course. These investigations were performed in strictly monitored growth conditions; environmental factors of light, temperature and humidity were kept uniform among chambers. Atmospheric composition in the chambers was kept at 0.05 kPa Carbon Dioxide, 21% Oxygen and a balance of Nitrogen in both experiments regardless of total chamber pressure. As was seen previously (Paul et al. 2004), there were no obvious morphological difference of plants grown on the surface of MS media plates between atmospheric conditions. All plants appeared similar in appearance to the 97 kPa control and even plants grown in extreme hypobaria (10 and 5 kPa) showed no physical signs of desiccation, such as wilting. Figure 2 provides representative images of plants after each treatment, just prior to being harvested to RNAlater. Figure 2A shows a representative plate of 10-day-old plants after 24 h of exposure to 97, 75, 50, 25, 10 and 5 kPa. Figure 2B shows a representative plate of 10 day old plants after being grown in either a 10 or a 97 kPa environment for 1, 3, 6, 12, 48 and 72 h. One additional atmospheric treatment was made at 0.7 kPa, a pressure that is comparable to what is typical on the surface of Mars. The plates of plants were observed in real time and then were allowed to recover and planted in soil. Figure 2C shows a picture of the plate of wilted plants after 24 h at 0.7 kPa (left), alongside a photograph of the transplanted seedlings 2 weeks after being transferred to soil (right). The plants recovered from the severe wilting imposed by 0.7 kPa and went on to flower and eventually set seed.

Overview of global transcriptional responses to different levels of hypobaria—experiment 1
The hypobaric transcriptomes of Arabidopsis roots and shoots were evaluated in response to 24 h of treatments in mild (75 kPa), moderate (50 kPa), severe (25 kPa) and extreme (10 and 5 kPa) low atmospheric pressures (Fig. 3). The genome-wide relative expression patterns

Table 2. The fluorescently tagged probes and paired primers used for qPCR.

| Gene Name    | Sequence (5'-3')       |
|--------------|------------------------|
| AHB1-Forward | GGTGCGCAAGTATGCATTGTT  |
| AHB1-Probe   | AGACGATAAAAGGAGCAGGCGGA|
| AHB1-Reverse | CCCCCAGCCCTCTATCCT     |
| PDC1-Forward | GCTCCTGGTACTGCTTCTC    |
| PDC1-Probe   | TCAAGAAGAAAAGGCCATCTGTGCA |
| PDC1-Reverse | TGGCCACAGTGATACGATCAG   |
| RD29A-Forward| TGTGCCGACGGGATTTG      |
| RD29A-Probe  | CGGAGAACAGATTTTCTTGCGGAAG |
| RD29A-Reverse| CTGATGCTACCCGTATCCA     |
| RD20-Forward | AAGCACAAGCGTGAGGCTG    |
| RD20-Probe   | TCGACGCATATACGACGCCAGAAAG |
| RD20-Reverse | TGTTCTCGAGGTTAACCTGGGACAT |
| UBQ11-Forward| AACTTGGAGGCGCCAGACCTTT  |
| UBQ11-Probe  | CAGAAAGGACTCTACGGGCTTTGTGTTG |
| UBQ11-Reverse| GTGATGGCTTTCCGGTCAAA     |

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Figure 2. Phenotypes of 10-day-old Arabidopsis grown in LPGC under multiple treatments. (A) Plants treated for 24 h in different atmospheric pressure levels. (B) Plants treated in 10 and 97 kPa for 72 h of time course. Photos in the time point of 6, 12 and 48 h are shown. (C) Plants treated for 24 h in 0.7 kPa (simulation of Martian atmospheric pressure) (Left). The phenotype of the same set of plants transplanted to soil for 2 weeks (Right). All photos were taken immediately before plants were fixed in RNAlater.
from plants in each hypobaric condition were compared to the reference transcriptome from plants grown in a chamber kept at ambient pressure (97 kPa). Unless noted otherwise, in all cases gene expression showing at least 2-fold change in abundance and $P < 0.01$ were defined as differentially expressed genes. A total of 3156 genes were differentially expressed by at least 2-fold in at least one hypobaric treatment, and yet not a single gene was co-ordinately expressed among all of the treatments in leaves, and only three genes showed coordinate expression in roots: POX1 (Proline dehydrogenase 1), PI4KG5 (Phosphatidylinositol 4-kinase gamma 5) and a gene (AT1G78850) that encodes a curculin-like lectin [see Supporting Information—Table S1].

For treatments at pressures between 75 and 10 kPa, the transcriptomes showed dramatic tissue-specific patterns; roots shared few differentially expressed genes in common with shoots in response to hypobaria.

Figure 3. Differential expression genes in response to 75, 50, 25, 10 and 5 kPa in roots and shoots. The heat map was plotted for 3156 differentially expressed genes with statistical significance ($P < 0.01$) by at least 2-fold in at least one condition of responses to hypobaria in roots or shoots. The scale bar indicates Log2 (fold change). Marker genes of desiccation and hypoxia response were labelled with arrows.
In addition, roots appeared more sensitive to these environmental changes, as far more genes were significantly changed in roots than in shoots (Figs 3 and 4A) [see Supporting Information—Table S1]. In the very extreme hypobaria of 5 kPa, there was more overlap in gene expression patterns between roots and shoots, with many more genes being differentially expressed in this environment than any other hypobaric treatment (Fig. 4A) [see Supporting Information—Table S1]. Supporting Information Table S1 provides a list of differentially expressed genes and GO terms of biological process in response to each hypobaric condition.

For roots, the differential expression patterns in 75 kPa were quite distinct when compared with other conditions. This mild hypobaria activated genes associated with transportation and localization processes, whereas moderate and severe hypobaria (50 kPa through 5 kPa) gene expression patterns were dominated by genes typically associated with abiotic, osmotic and oxidative stress responses, and the abundance of differentially expressed genes in these functional categories increased with the severity of the hypobaric environment. This trend is particularly evident in genes associated with hypoxia, such as SUS1, SUS4, PDC1, PDC2, AHB1, ERF073,

![Figure 4. Differential expression gene numbers in each response to hypobaria in roots and shoots. The overlap of differentially expressed genes between roots and shoots in each hypobarcic response are shown in venn diagrams (A) and numbers of differentially expressed genes with changes of >2-fold, >4-fold, >8-fold and 16-fold are exhibited in histograms for roots (B) and shoots (C).](image-url)
were not significantly affected in other pressures (Fig. 3) cited huge changes including hundreds of genes that were also induced in shoots in 5 kPa (PP2CA, which was only a small overlap (Fig. 4B) [see Supporting Information—Table S1].

Most of the genes that are repressed in hypobaric environments are associated with growth and metabolism. The mild hypobaria of 75 kPa resulted in more down-regulated genes than 50 and 25 kPa, among which there was only a small overlap (Fig. 4B) [see Supporting Information—Table S1].

In shoots, many of the differentially expressed genes at 50 kPa are associated with transport functions (e.g. LTH7, CAX3, STP13 and SWEET10) and pathogen response (e.g. TH12, WRKY18 and TAT3) rather than hypoxia. Below 50 kPa, the induction of genes associated with desiccation and water deprivation responsive pathways predominated. The Dehydration-Responsive Element-Binding Factor 2A (DREB2A) (Sakuma et al. 2006) as well as marker target genes RD29A, KIN1, COR47, COR15a, COR15b and ERD10 (Kasuga et al. 1999; Seki et al. 2002; Kimura et al. 2003) were induced by hypobaric environments of 10 kPa and below in shoots, but not by less severe hypobaria (Fig. 3). In addition, several members of PYL ABA receptor family were also involved in the effects of extreme hypobaria (Fig. 3) [see Supporting Information—Table S1]. For instance, PYL5 and PYL8 expression levels were significantly enhanced in roots in both 10 and 5 kPa and repressed in shoots in 5 kPa. An examination of genes that are induced by ABA in guard cells (Leonhardt et al. 2004) reveals many of which were also induced in shoots in 5 kPa (PP2CA, AHB12 and XERO2). Compared with 10 kPa, 5 kPa elicited huge changes including hundreds of genes that were not significantly affected in other pressures (Fig. 3) [see Supporting Information—Table S1].

Time course-dependent transcriptome analysis revealed an organ specific pattern in response to extreme hypobaric stress—experiment 2

The atmospheric environment of 10 kPa was chosen to evaluate the progression of the physiological adaptation to hypobaria. Transcriptome analyses of 10-day-old plants (at start of the experiment) exposed for 1, 3, 6, 12, 48 and 72 h revealed organ-specific and time point dependent patterns of gene expression (Fig. 5). Since the 10 kPa, 24 h treatment was represented in Experiment 1, this treatment was not included in Experiment 2. There were a total of 2801 genes that were differentially expressed in at least one time point over the course of the 3-day experiment. Only 200 of those genes (7%) were coordinately expressed with similar trends in both roots and shoots [see Supporting Information—Table S2]. The consistently up-regulated genes included typical hypoxia responsive genes such as ADH1, AHB1, PDC1, PDC2, PCO1 and PCO2 (Chang and Meyerowitz 1986; Gibbs et al. 2011; Gasch et al. 2016) as well as drought and cold marker genes like RD29A and KIN1 (Yamaguchi-Shinozaki and Shinozaki 1994; Wang and Cutler 1995; Wang et al. 1995; Narusaka et al. 2003).

In clustering analysis, Kendall tau distances of gene expression between each time point were used to show the similarity of transcriptome responses in time course. In roots, the patterns were distinctly different in each of the early time points (1 and 3 h), but then a trend developed at 6 h that was continued through 72 h. In shoots, it was the earlier time points that exhibited similar patterns of gene expression (from 1 to 12 h), while in the later time points new sets of genes were either induced or repressed (Fig. 6A). The relative intensity of the response was gauged by the number of differentially expressed genes, and their relative increase or decrease at each time point. In roots, there appears to be a peak of differential expression at 12 h, with a relatively smooth increase approaching 12 h and then a decline approaching the 72 h point (Fig. 6B). In shoots, there is not a clear trend, other than the overall number of differentially expressed genes tends to increase over time (Fig. 6C).

Roots respond more quickly than shoots, as demonstrated by the large number of induced genes at 1 h. In contrast, the intensity of the response appears to generally mount over time in shoots, particularly with respect to down-regulated genes. The early response in roots could be promoted by the hypoxic component of hypobaria; the plant low-oxygen response consists of approximately two stages in a previous time course study, 0–0.5 h and 2–20 h (Klok et al. 2002). Indeed, some key hypoxia responsive genes were induced and kept active while other stress responders like WRKY6 (Skibbe et al. 2008; Chen et al. 2009) and WRKY21 (Phelps-Durr et al. 2005) were transiently induced at 1 h. At time point of 3 h, cell wall organization and cell growth associated genes such as gibberellin biosynthesis gene GA3ox1 (Arnaud et al. 2010) were transiently repressed. Metabolic transportation and localization related genes were induced at 6 h and kept active through the 72 h time point. From 12 to 72 h the cellular metabolic processes appeared to be continually altered, as for each time point a unique set of genes were differently expressed within this functional category (Figs 5 and 6B).

Shoots exhibited fewer differentially expressed genes in the early time points than roots. Compared to roots, shoots also have fewer examples of genes which display a mounting degree of expression over time. Nonetheless,
there were many genes associated with biosynthetic and metabolic processes that were induced or repressed early, and maintained or exceeded those levels of expression over time (Figs 5 and 6C). At 48 h, additional water stress and ABA responsive genes such as PP2C family members HAI1, ABI2, and homeodomain leucine-zipper gene ATHB7 (Soderman et al. 1996; Merlot et al. 2001; Olsson et al. 2004; Yoshida et al. 2006) were involved to cope with hypobaria. Finally at 72 h, stress response was down-regulated and nitrogen compound metabolic process was induced [see Supporting Information—Table S2].

In pathway enrichment investigation, the scheme of metabolic changes in roots and shoots could be further exhibited (Fig. 7). Different from the GO analysis using time course-dependent group of genes shown in Supporting Information—Table S2, Fig. 7 shows the KEGG pathways significantly enriched in all differentially expressed genes (Benjamini corrected p-value < 0.05) of each time point. Metabolic changes widely happened in
both two tissues. Roots showed more similar metabolites biosynthesis process before 12 h, and from 12 to 72 h nitrogen and hormone associated signalling were identified. In shoots, no pathway was detected in 1 h, and multiple secondary metabolites associated signalling changed after 12 h (Fig. 7).

Validation of array data using qRT-PCR
In this study, we used unadjusted $P$ value to perform differential analysis, for the adjustment such as FDR would hide all differentially expressed genes in some comparisons. For validation of array data, two hypoxia associated genes AHB1 (AT2G16060) and PDC1 (AT4G33070) as well as two drought associated genes RD29A (AT5G52310) and RD20 (AT2G33380) were subjected to qRT-PCR analysis. Most of the expression changes showed an agreement between qRT-PCR and RNA-seq data (Fig. 8). AHB1 and PDC1 with $P$ values < 0.01 but with FDR > 0.05 in the arrays exhibited significant changes in 50 kPa in roots, indicating that FDR indeed limited the identification of differentially expressed genes in our data. Similarly, the desiccation responsive genes RD29A and RD20 were highly up-regulated in shoots in responses to extreme hypobaric stress.

Discussion
It is known that plants survive and thrive in hypobaric environments according to the extensive studies that have focused on direct physiological changes and metabolic impact of low atmospheric pressures on plants (Rule and Staby 1981; Musgrave et al. 1988; Andre and Massimino 1992; Daunicht and Brinkjans 1996; Corey et al. 2002; Spanarkel and Drew 2002; He et al. 2003). Data presented here suggest, however, that plants mount an extensive and complex stress response at the level of gene expression in order to drive that survival—a response that is not linear with respect to increasing severity, and has dramatic organ specificity. This stress response profile seems contradictory to the apparent ability of plants to thrive at low pressures, and allows an examination of the value and appropriateness of the responses to the novel stress of hypobaria.

Two highly recognizable stressors of hypobaric environments are the reduction of available oxygen and elevation of evapotranspiration rates (Paul et al. 2004; Rygalov et al. 2004). Genes associated with both hypoxic stress and drought responses are highly represented in even mild hypobaria. However, while mitigating the hypoxia characteristic of hypobaric environments makes metabolic sense, engaging extensive drought responses is inconsistent with the well-watered and humid environment in these experiments. Therefore, the drought responses may be a metabolically inappropriate reaction and therefore present an unnecessary metabolic cost in low pressure environments, especially the mild and moderate pressures of 25 kPa and above.

In terms of atmospheric pressure, the habitable terrestrial limit for angiosperms converges on about 50 kPa (Körner 2003a; Klimes 2003; Namgail et al. 2012), a value that is driven by the limits of habitable altitudes in mountains of neo-tropical latitudes, such as the Himalayas. The physical terrestrial limit (as in ground to stand on) of atmospheric pressure is 34 kPa, which can be found at the top of Mt. Everest. In natural habitats, limits are primarily driven by combinations of extreme temperature and water stress, rather than by atmospheric pressure.
However, in laboratory chambers the harsh environmental characteristics that attend high altitudes can be mitigated (75 and 50 kPa), and truly novel pressure environments (25, 10, and 5 kPa) can be created that are survivable by terrestrial plants. These capabilities allowed us to explore the fundamental limits of plant responses to novel environments, but also to examine the mechanisms that underlie coping with only the hypobaric component of high altitude environments.

The degree of hypobaria greatly influences the transcriptional response of plants, but the response is not linear. Of the more than 3000 genes that are differentially expressed among the five hypobaric treatments (Fig. 2) [see Supporting Information—Table S1], only three in roots (POX1, PI4KG5 and an unknown D-mannose binding lectin protein) and two in shoots (PCR1 and GSTF6) are coordinately induced across all hypobaria conditions. These genes may play basic roles in plant hypobaric responses although little is known about their molecular functions. The primary reason behind the virtual absence of coordinately expressed genes among treatments is that the gene expression profile of the 75 kPa treatment had virtually nothing in common with hypobaric treatments of 50 kPa and lower. If the 75 kPa data are considered separately from the other treatments, then a pattern begins to emerge from the transcriptional response to increasingly severe hypobaric exposure.

Increasing the severity of hypobaric environments

Although 75 kPa would seem to be simply a more benign environment than the other atmospheres, there are characteristics of 75 kPa that set this pressure apart from the rest. Of the five treatments, 75 kPa is the only pressure that represents a hypobaric environment that can be part of a biologically rich terrestrial habitat. In other words, 75 kPa does not present a novel environment that is outside the evolutionary experience of higher plants; it can be found on the slopes of a 3000 m mountain. In addition, the combination of reduced O2 and CO2 partial pressures, reduced boundary layer resistance and increased gas diffusion rates, can increase photosynthetic efficiency in plants grown at 75 kPa (He et al. 2013; Richards et al. 2006; Rygalov et al. 2004), and change the efficacy hormones such as ethylene (Apelbaum and Burg 1972; Burg and Burg 1965). Further, although effects vary widely among plant species and habitats, there is ample evidence that photosynthetic efficiency is influenced by altitude in natural hypobaric environments (Gale 1972, 1973, 2004; Shi et al. 2006). Thus, the evolutionarily familiar features of the 75 kPa environments engage metabolic processes that are familiar and reasonably well understood.

In the mild hypobaria of 75 kPa, a common theme among the most highly induced genes in roots was the
over expression of genes encoding transcription factors and genes associated with root development or cell growth. Most of the highly down regulated genes in roots are associated with light signalling and the photosystems, while very few are typically associated with stress metabolism. In shoots, there was an abundance of differentially expressed genes associated with oxidative stress and pathogen responses, as well as many genes encoding transporters. Many of the down regulated genes are associated with cell growth and elongation.

In contrast to the familiar and well understood responses at 75 kPa, responses to 50 kPa are distinctly unfamiliar and seemingly illogical. The transition to 50 kPa marks the limits of terrestrial plant environments, yet although this is a harsher hypobaric treatment, plants respond with a decrease in the number of differentially expressed genes (112 in roots and 23 in shoots) compared to the 75 kPa response (196 in roots and 60 in shoots). More interestingly, the type of genes that are expressed at 50 kPa are quite different than those at 75 kPa.

Figure 8. Confirmation of gene expression profiles using qPCR. The transcript levels of PDC1 (AT4G33070), AHB1 (AT2G16060), RD29A (AT5G52310) and RD20 (AT2G33380) were determined by Taqman quantitative RT-PCR for RNA samples from the same 10-day-old root and shoot tissues used for microarray analysis. The UBQ11 (AT4G05050) was used as the internal control. The blue bars present Log2 fold-change of expression level relative to 97 kPa control for each sample in qPCR. Data are means ± SE (n = 3, *P < 0.05, **P < 0.01). Orange bars present Log2 fold-change of expression level relative to 97 kPa control for each sample in microarray (*P < 0.01, **P < 0.001).
and they represent a developing pattern of response that is carried though with increasing severity of hypobaria, particularly in roots. Figure 3 provides a sense of this transition pattern. About a third (35) of the differentially expressed genes at 50 kPa in roots are coordinately expressed in 25, 10 and 5 kPa. Most are upregulated, and many are indicative of hypoxic stress.

Below 50 kPa, decreasing the atmospheric pressure correlates directly with an increase in the number of differentially expressed genes in an organ-specific manner. In roots there is substantial (60–70%) overlap of the genes that are induced in each step of severity, giving the impression that the root response to increasingly severe hypobaria builds on a common metabolic foundation (Fig. 2) [see Supporting Information—Table S1]. In roots, it appears that a consistent core of the response to increasingly reduced atmospheric pressure is an induction of genes associated with hypoxic stress.

In shoots, the patterns of gene differential gene expression are more varied among hypobaric treatments, and hypoxic stress associated genes are less abundant than in roots at the same pressure. One of the mitigating effects of hypoxic stress in shoots is the presence of photosynthesis (e.g. Juntawong and Bailey-Serres 2012). The largest category of differentially expressed genes in shoots from 10 and 5 kPa are those associated with desiccation and ABA signalling related processes. For instance, in the top 25 most highly induced genes in 10 kPa, almost a third fall into this category (e.g. LTP3, LTP4, LEA7, BG2, PXG3, COR15A, COR15B and STR17).

But are hypobaric plants ‘drought stressed’ from a water deficiency? The plants were grown in a humid environment (>95% rh within the plates), on a medium that provided a continuous supply of water, and although fresh weights were not collected from treatment and control representatives in these plants, the plants did not show any outward signs of dehydration stress, such as wilting. This observational conclusion is supported by fresh weight data collected in an earlier experiment, in which there was no significant change in average fresh weight between hypobaric and control Petri plate-grown plants after 24 h at 10 kPa (Paul et al. 2004). Thus it is likely that the desiccation response is due to the perception of increased water flux caused by the low pressure environment rather than the absolute loss of water in the plants (Corey et al. 2002; Paul et al. 2004). Correspondingly, the drought response is related to the rate of water movement through the leaves, as a few of key desiccation associated genes including RD29a, COR15a and KIN1 (Kasuga et al. 1999) were only induced in response to extreme hypobaric stress (below 25 kPa). The sense of water stress by plants in mild, moderate and even severe low atmospheric pressure around 25 kPa can be quite weak. In addition, there is a marked difference in the induction of drought-induced genes in roots as compared with shoots. Although some of the hallmark genes (e.g. COR78 and COR15A (Horvath et al. 1993; Wilhelm and Thomashow 1993) are also induced by cold (Zhou et al. 2011), the profiles in leaves and roots are more closely aligned in response to drought stress.

Increasing the duration in a severe of hypobaric environment

The 10 kPa hypobaric environment was selected to explore the molecular processes associated with the physiological adaptation of Arabidopsis to extreme hypobaria over time. Plants grown at 10 kPa for 24 h present a strong transcriptional response, and distinct organ-specific patterns of gene expression in response to hypobaria (Fig. 5). The time course was constructed to characterize early responses (1, 3, 6 and 12h of exposure) and then longer (48 and 72 h) to discover responses that may represent an approach to homeostasis.

Using clustering and pathway enrichment analysis, we observed that roots and shoots underwent different stages in the duration of hypobaric treatment. These gene expressions provided a preliminary look into how plants physiologically adapt to the novel environment of 10 kPa. Initially, both roots and shoots appeared to adjust basic metabolic processes; enhancing carbohydrate metabolism while aspects of growth and development. It appeared to take about 72 h in 10 kPa for plants to have stabilized many of the initially adjusted metabolic processes, as many of the early onset gene inductions and repressions were no longer differentially expressed. After 72 h, there was an increase in differential expression of genes associated with surviving aspects of drought and other abiotic stress, particularly hypoxia, which was consistent with the impact of the hypoxic component of hypobaria. However, there were distinct differences in how roots and shoots each responded to hypobaria over time [see Supporting Information—Table S2].

In roots, although the overall expression patterns of 1 and 3 h were comparatively distinct and the transcription time points after 6 h were more closely aligned (Fig. 6A), the 12 h point in the time course appeared to mark a boundary for a different set of metabolic pathways (Fig. 7). The initial response in roots appeared to primarily adjust in numerous metabolic processes such as glycolysis changes, which is a typical hypoxia related metabolic alteration (Liu et al. 2005). After 12 h, gene expression patterns suggested an increase in plant hormone signalling changes while many processes associated with growth...
and development were negatively regulated [see Supporting Information—Table S2].

In shoots, the gene expression patterns from 1 to 12 h were more similar while the late stage of 48 and 72 h showed distinct patterns (Figs 5 and 6A). Accordingly, the metabolic pathway identification was in line with the 12 h time point of boundary (Fig. 7). From the onset of hypobaria to 12 h, gene expression patterns suggested that shoots were engaging multiple stress responses while increasing cellular metabolism such as alpha-Linolenic acid metabolism. At later stages, the abundance of differentially expressed genes associated with desiccation and drought signal transduction further suggested that plants were attempting to physiologically adapt to perceived desiccation. In summary, the time point of 12 h can be a turning point of plant metabolism in responses to extremely low atmospheric pressure.

Although the transcriptomic response of roots and shoot to each hypobaric time point were mostly distinct with respect to specific genes, there were several cases where different members of the same gene family were engaged in an organ-specific manner. In other words, although the exact same genes were not coordinately expressed across the board in both roots and shoots, representatives of the same gene family were engaged in both organs. Two interesting examples are the PLY family of ABA receptors, and the XTH family of cell wall remodelling genes.

The PYL family of ABA receptors serve to play a major role in ABA signalling required for vegetative and reproductive growth such as quantitative regulation of stomatal apertures in leaves, and yet the 14 members of the family can have distinct functional differences (Gonzalez-Guzman et al. 2012). In roots, receptor components PYL2, PYL5, PYL6 and PYL8 were up-regulated especially in early stage (3 and 6 h), while PYL1 was down-regulated along every point of time course. However, in shoots, the genes encoding PYL5 and PYL7 were down-regulated in early stage (before 6 h). The down-regulation of PYL5 and PYL7 in shoots seems to run counter to a hypobaric adaptive response as it should render the plants more sensitive to water loss by increasing the stomata aperture, and thereby enhance the rate of hypobaria-induced water movement from the plant. However, it is possible that roots are inducing PYL’s as part of a plant-wide ‘drought’ response interpreted from the elevated water movement through the stomata, but that additional sensing mechanisms (such as turgor receptors) provide feedback in leaves that influences gene expression in the leaves.

The XTH family of enzymes function in xyloglucan endotransglycosylation (XET) and xyloglucan hydrolysis (XEH) to regulate chain length and modifications of xyloglucans. Xyloglucans are hemicellulose structural molecules that coat and cross-link cellulose fibers in the primary cell wall and are particularly important in maintaining the structural integrity to withstand high turgor pressure within cells conducting water (such as phloem sieve tubes) (Bourquin et al. 2002) and have a wide a varied distribution among plant organs (Yokoyama and Nishitani 2001). Nineteen members of the XTH family were differentially expressed in response to hypobaria in both roots and shoots: XTH5, XTH7, XTH8, XTH12, XTH13, XTH14, XTH15, XTH16, XTH17, XTH18, XTH19, XTH20, XTH21, XTH22, XTH23, XTH24, XTH25, XTH30 and XTH31, but in widely differing patterns. In roots, XTH7, XTH12, XTH13, XTH14, XTH16, XTH17 and XTH21 were generally downregulated, and XTH5, XTH18, XTH20, XTH22 and XTH23 were generally upregulated. A few were varied in their expression patterns across the time course (XTH15 and XTH24). In shoots, most of the differentially expressed XTH’s were down regulated after 6 h at 10 kPa (XTH4, XTH8, XTH15, XTH19, XTH25 and XTH30). The exception was XTH31, which was upregulated after 3 h, in contrast to what was seen in roots for XTH31.

The differences between root and shoot utilization implied that each organ was using diverse cell wall metabolic tools, respectively, to sense and respond to the extreme hypobaria. The enzymes that regulate features of these structural molecules are of particular interest because of the flexibility they can confer in physiological adaptation to stress by providing a mechanism to regulate cell wall expansion and structural integrity in response to environmental changes (Rose et al. 2002; Eklof and Brunner 2010). It is possible that this family of cell wall remodelling genes plays a substantial role in the rapid adjustment of cell wall structure to accommodate changes in the environment, particularly those associated with water stress.

Interestingly, examples from the extended family of cell wall remodelling genes are also abundant in the differentially expressed genes in response to another novel environment—spaceflight. There have been several recent spaceflight transcriptome studies that have shown that genes associated with cell wall remodelling are important to the physiological adaptation to this novel environment as well (Paul et al. 2012, 2013; Correll et al. 2013; Sugimoto et al. 2014, Kwon et al. 2015). It is possible that the cell wall, as the first line of defence for a plant cell, is also the first responder to environments that present a novel stimulus. Both spaceflight and hypobaria may engage sensing mechanisms that can be interpreted as effecting a breach or a loosening of cell wall structures, which in turn engage a variety of genes that typically target pathogen responses and mechanical wounding. While both hypobaric and spaceflight
Conclusions
The unique differential gene expression patterns in response to hypobaria suggest that the physiological adaptation of Arabidopsis to reduced atmospheric pressure is more complex than the acclimation to the reduced partial pressures of oxygen inherent to low atmospheric pressures. Arabidopsis plants respond to hypobaria with a variety of changes in gene expression patterns that are organ specific, change with respect to the severity of the hypobaric environment, and adjust over time. The hypobaric response appears to overlay the perception of desiccation and biotic challenge on top of the perceptions of hypoxic stress, which suggests that the practical compensation for hypobaric stress in plants cannot be as simple as increasing the oxygen content, as is done for humans in the low pressure environments of space vehicles and space suits, or on the top of Mt. Everest (Paul and Ferl 2006).

These studies provide a window into the nature of the strategies used for physiological adaptation to hypobaria as an example of novel environments, and can allow dissection of specific components of plant responses to hypobaria as part of space exploration mission habitat design. For example, in a well hydrated, yet hypobaric greenhouse on Mars, plants would not need to expend the metabolic energy to maintain the drought response the hypobaria would elicit. Thus, there could be an advantage to engineer the plants along with the habitats envisioned for planetary exploration. The more fundamental application of this physical phenomenon is that plant metabolic responses to drought stress and stomatal regulation can be examined without subjecting the plant to comprehensive desiccation.

In addition, these transcriptome analyses may reveal genes that have contributed to the natural adaptation of plants to high altitude environments. It has been argued that plants that have evolved to live in a ‘stressful’ environment are not actually ‘stressed’ at all, rather, this is the habitat that is normal to them, and so the metabolic processes employed are just as benign as those engaged by their cousins living in seemingly more hospitable climates (e.g. Körner 2003b). Indeed, many of the differentially expressed genes at 75 kPa (e.g. genes encoding gibberellin-regulated proteins, xyloglucan endotransglucosylase/hydrolases, glutathione s-transferases, dehydrins and expansins) are also differentially regulated in high altitude species and ecotypes when compared to representatives indigenous to lower altitudes (e.g. Chapman et al. 2013; Dogra et al. 2016; Luo et al. 2015).

As a unique stress, hypobaria presents an opportunity to understand the development of plant responses to novel environmental stress. We conclude that some aspects of the overall response to hypobaria are appropriate, in that hypoxia responses to the low oxygen of hypobaria make sense. However, we also conclude that some elements of the response are inappropriate, in that the drought responses that are seen in hypobaria are inconsistent with a wet and humid environment. These seemingly inappropriate responses may help illuminate drought perception mechanism in plants, and further suggest strategies for improving growth in hypobaria especially as pressure is considered in extraterrestrial habitats and vehicles.

Supporting Information
The following additional information is available in the online version of this article—

Table S1. Full list of differentially expressed genes in response to 75 kPa, 50 kPa, 25 kPa, 10 kPa and 5 kPa in roots and shoots of 10-day-old plants. There are 3156 genes that present significant (p < 0.01) differential expression by at least 2-fold in at least one condition (including 75 kPa vs 97 kPa, 50 kPa vs 97 kPa, 25 kPa vs 97 kPa, 10 kPa vs 97 kPa and 5 kPa vs 97 kPa in roots or shoots). The differentially expressed genes are categorized according to log value of fold change and GO terms of biological process are listed for each gene clade.

Table S2. Full list of differentially expressed genes in response to 10 kPa during a time course in roots and shoots of 10-day-old plants. There are 2801 genes that present significant (p < 0.01) differential expression by at least 2-fold in at least one condition (including 10 kPa for 1 h vs 97 kPa for 1 h, 10 kPa for 3 h vs 97 kPa for 3 h, 10 kPa for 6 h vs 97 kPa for 6 h, 10 kPa for 12 h vs 97 kPa for 12 h, 10 kPa for 48 h vs 97 kPa for 48 h and 10 kPa for 72 h vs 97 kPa for 72 h in roots or shoots). The differentially expressed genes are categorized according to log value of fold change and GO terms of biological process are listed for each gene clade.

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Contributions by the Authors
A.-L.P. and M.Z. contributed equally to the manuscript. A.-L.P. and M.Z. performed the data analysis, and took the lead on manuscript development. A.-L.P, R.J.F., J.B.C., M.R. and M.S. prepared plant materials and conducted the low atmospheric pressure treatments at the University of Guelph. A.R. carried out statistical analysis of array data. A.K.Z. contributed to data analyses and was responsible for data archiving in GEO. M.A.D. is the director of the University of Guelph Controlled Environment Systems Research Facility, and contributed to the experimental design. R.J.F. and A.-L.P. were responsible for the overall experimental design and conduct of the experiments. All authors read and approved the final manuscript.

Conflict of Interest Statement
None declared.

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Literature Cited
Andre M, Massimino D. 1992. Growth of plants at reduced pressures: experiments in wheat-technological advantages and constraints. Advances in Space Research 12:97–106.
Apelbaum A, Burg SP. 1972. Effects of ethylene and 2,4-dichlorophenoxyacetic acid on cellular expansion in Pisum sativum. Plant Physiology 50:125–131.
Arnaud N, Girin T, Sorefan K, Fuentes S, Wood TA, Lawrenson T, Sablowski R, Ostergaard L. 2010. Gibberellins control fruit patterning in Arabidopsis thaliana. Genes and Development 24:2127–2132.
Bailey-Serres J, Chang R. 2005. Sensing and signalling in response to oxygen deprivation in plants and other organisms. Annals of Botany 96:507–518.
Bourquin V, Nishikubo N, Abe H, Brumer H, Denman S, Eklund M, ChristierMN, Teeri TT, Sundberg B, Mellerowicz EJ. 2002. Xyloglucan endotransglycosylases have a function during the formation of secondary cell walls of vascular tissues. The Plant Cell 14:3073–3088.
Burg SP, Burg EA. 1965. Ethylene action and the ripening of fruits. Science 148:1190–1196.
Cai J, Keen HL, Sigmund CD, Casavant TL. 2012. Coex-Rank: an approach incorporating co-expression information for combined analysis of microarray data. Journal of Integrative Bioinformatics 9:208.
Chang C, Meyerowitz EM. 1986. Molecular cloning and DNA sequence of the Arabidopsis thaliana alcohol dehydrogenase gene. Proceedings of the National Academy of Sciences of the United States of America 83:1408–1412.
Chapman MA, Hiscock SJ, Filatov DA. 2013. Genomic divergence during speciation driven by adaptation to altitude. Molecular Biology and Evolution 30:2553–2567.
Chen YF, Li LQ, Xu Q, Kong YH, Wang H, Wu WH. 2009. The WRKY transcription factor modules PHOSPHATE1 expression in response to low Pi stress in Arabidopsis. The Plant Cell 21:3554–3566.
Clawson JM, Hoehn A, Stodieck LS, Todd P. 1999. The integration of existing technologies for efficient, affordable space flight agriculture. SAE Paper No. 1999-1-2176. In 29th International Conference on Environmental Systems (ICES), Denver, CO.
Corey KA, Barta DJ, Wheeler RM. 2002. Toward Martian agriculture: responses of plants to hypobaria. Life Support and Biosphere Science 8:103–114.
Corey KA, Bates ME, Adams SL. 1996. Carbon dioxide exchange of lettuce plants under hypobaric conditions. Advances in Space Research 18:265–272.
Correll MJ, Pyle TP, Millar K, Sun Y, Yao J, Edelmann RE, Kiss JZ. 2013. Transcriptome analyses of Arabidopsis thaliana seedlings grown in space: implications for gravity-responsive genes. Planta 238:519–533.
Daunicht HJ, Brinkjans HJ. 1996. Plant responses to reduced air pressure: advanced techniques and results. Advances in Space Research 18:273–281.
Dogra V, Sharma R, Yelam S. 2016. Xyloglucan endo-transglycosylase/hydrolase (XET/H) gene is expressed during the seed germination in Podophyllum hexandrum: a high altitude Himalayan plant. Planta 244:505–515.
Du Z, Zhou X, Ling Y, Zhang Z, Su Z. 2010. AgriGO: a GO analysis tool-kit for the agricultural community. Nucleic Acids Research 38:W64–W70.
Eisen MB, Spellman PT, Brown PO, Botstein D. 1998. Cluster analysis and display of genome-wide expression patterns. Proceedings of the National Academy of Sciences of the United States of America 95:14863–14868.
Eklof JM, Brumer H. 2010. The XTH gene family: an update on enzyme structure, function, and phylogeny in xyloglucan remodeling. Plant Physiology 153:456–466.
Fowler PA, Wheeler RM, Bucklin RA, Corey KA. 2000. Low pressure greenhouse concepts for Mars. Inflatable Greenhouse Workshop. NASA TM 2000-208577:116-123.
Gasch P, Mudinger M, Muller JT, Lee T, Bailey-Serres J, Mustroph A. 2016. Redundant ERF-VII transcription factors bind to an evolutionarily conserved cis-motif to regulate hypoxia-responsive gene expression in Arabidopsis. The Plant Cell 28:160–180.
Gibbs DJ, Lee SC, Isa NM, Gramuglia S, Fukao T, Bossel GW, Correia CS, Corbineau F, Theodoulou FL, Bailey-Serres J, Holdsworth MJ.
2011. Homeostatic response to hypoxia is regulated by the N-end rule pathway in plants. *Nature* **479**:415–418.

Gonzalez-Guzman M, Plizio GA, Antoni R, Vera-Sirena F, Merilo E, Bossel GW, Fernández MA, Holdsworth MJ, Perez-Amador MA, Kollist H, Rodriguez PL. 2012. Arabidopsis PYR/PYL/RCAR receptors play a major role in quantitative regulation of stomatal aperture and transcriptional response to abscisic acid. *The Plant Cell* **24**:2483–2496.

Goto E, González-Guzman M, Pizzio GA, Antoni R, Vera-Sirera F, Merilo E, Le Gall H, Philippe F, Domon JM, Gillet F, Pelloux J, Rayon C. 2015. Ethylene reduces plant gas exchange and growth of lettuce grown from seed to harvest under hypobaric and ambient total pressure. *Journal of Plant Physiology* **186**:369–378.

Hernández-Vázquez D, Cisneros-Zevallos L, Davies FT Jr. 2013. Hypobaria and hypoxia affects phytochemical production, gas exchange, and growth of lettuce plants under hypobaric and normal atmospheric conditions. *Physiologia Plantarum* **133**:258–271.

He C, Davies FT Jr, Lacey RE. 2007. Separating the effects of hypobaria and hypoxia on lettuce: growth and gas exchange. *Physiologia Plantarum* **131**:226–240.

He C, Davies FT Jr, Lacey RE. 2009. Ethylene reduces gas exchange and growth of lettuce plants under hypobaric and normal atmospheric conditions. *Physiologia Plantarum* **135**:258–271.

He C, Davies FT Jr, Lacey RE. 2003. Effect of hypobaric conditions on ethylene evolution and growth of lettuce and wheat. *Journal of Plant Physiology* **160**:1341–1350.

He C, Jacobo-Velázquez D, Cisneros-Zevallos L, Davies FT. 2013. Hypobaria and hypoxia affects phytochemical production, gas exchange, and growth of lettuce. *Photosynthetica* **51**:465–473.

Horvath DP, McLarney BK, Thomashow MF. 1993. Regulation of *Arabidopsis thaliana* L. (Heyn) cor78 in response to low temperature. *Plant Physiology* **103**:1047–1053.

Juntaowong P, Bailey-Serres J. 2012. Dynamic light regulation of translation status in *Arabidopsis thaliana*. *Front Plant Sci* **3**:66.

Kasuga M, Liu Q, Miura S, Yamaguchi-Shinozaki K, Shinozaki K. 1999. Improving plant drought, salt, and freezing tolerance by gene transfer of a single stress-inducible transcription factor. *Nature Biotechnology* **17**:287–291.

Kimura M, Yamamoto YY, Seki M, Sakurai T, Sato M, Abe T, Yoshida S, Manabe K, Shinozaki K, Matsui M. 2003. Identification of Arabidopsis genes regulated by high light-stress using cDNA microarray. *Photochemistry and Photobiology* **77**:226–233.

Klimes L. 2003. Life-forms and clonality of vascular plants along an altitudinal gradient in E Ladakh (NW Himalayas). *Basic and Applied Ecology* **4**:317–328.

Klok EJ, Wilson IW, Wilson D, Chapman SC, Ewing RM, Somerville SC, Pecock WJ, Dolferus R, Dennis ES. 2002. Expression profile analysis of the low-oxygen response in Arabidopsis root cultures. *The Plant Cell* **14**:2481–2494.

Körner C. 2003a. *Alpine plant life*, 2nd edn. Heidelberg: Springer.

Körner C. 2003b. Limitation and stress: always or never? *Journal of Vegetation Science* **14**:141–143.

Kwon T, Sparks JA, Nakashima J, Allen SN, Tang Y, Blancaflor EB. 2015. Transcriptional response of Arabidopsis seedlings during spaceflight reveals peroxiside and cell wall remodeling genes associated with root hair development. *American Journal of Botany* **102**:21–35.

Le Gall H, Philippe F, Domon JM, Gillet F, Pelloux J, Rayon C. 2015. Cell wall metabolism in response to abiotic stress. *Plants (Basel)* **4**:112–166.
Paul A-L, Schuerger AC, Popp MP, Richards JT, Manak MS, Ferl RJ. 2004. Hypobaric biology: Arabidopsis gene expression at low atmospheric pressure. *Plant Physiology* **134**:215–223.

Paul A-L, Zupanska A, Ostrow DT, Zhang Y, Sun Y, Li J-L, Shanker S, Farmerie WG, Amalfitano CE, Ferl RJ. 2012. Spaceflight transcriptomes: unique responses to a novel environment. *Astronomy* **12**:40–56.

Paul A-L, Zupanska AK, Schultz ER, Ferl RJ. 2013. Organ-specific remodeling of the Arabidopsis transcriptome in response to spaceflight. *BMC Plant Biology* **13**:112.

Ritchie ME, Phipson B, Wu D, Hu Y, Law CW, Shi W, Smyth GK. 2015. Limma powers differential expression analyses for RNA-sequencing and microarray studies. *Nucleic Acids Research* **43**:e47.

Rule DE, Staby GL. 1981. Growth of tomato seedlings at sub-atmospheric pressures. *HortScience* **16**:331–332.

Salman DA. 2004. Java Treeview: extensible visualization of microarray data. *Bioinformatics* **20**:3246–3248.

Sambrook J, Fritsch EF, Maniatis T. 1989. *Molecular Cloning: A Laboratory Manual*. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

Shinozaki K, Yamaguchi-Shinozaki K. 1994. A novel cis-acting element in an Arabidopsis gene is involved in responsiveness to drought, low-temperature, ABA, osmoticum and dehydration. *Plant and Cell Physiology* **35**:271–276.

Spanarkel RB, Jackson MB. 1997. Plant adaptations to anaerobic stress. *Annuals of Botany* **79**:1–20.

Wang H, Cutler AJ. 1995. Promoters from kin1 and cor6.6, two Arabidopsis thaliana low-temperature- and ABA-inducible genes, direct strong beta-glucuronidase expression in guard cells, pollen and young developing seeds. *Plant Molecular Biology* **28**:619–634.

Wang H, Datla R, Georges F, Loewen M, Cutler AJ. 1995. Promoters from kin1 and cor6.6, two homologous Arabidopsis thaliana genes: transcriptional regulation and gene expression induced by low temperature, ABA, osmoticum and dehydration. *Plant Molecular Biology* **28**:605–617.

Wieland PO. 1998. Living together in space: the design and operation of the life support systems on the International Space Station. NASA TM-1998-206956 1.

Wilhelm KS, Thomashow MF. 1993. Arabidopsis thaliana cor15b, an apparent homologue of cor15a, is strongly responsive to cold and ABA, but not drought. *Plant Molecular Biology* **23**:1073–1077.

Yamaguchi-Shinozaki K. 1994. A novel cis-acting element in an Arabidopsis gene is involved in responsiveness to drought, low-temperature, or high-salt stress. *The Plant Cell* **6**:251–264.

Yokoyama R, Nishitani K. 2001. A comprehensive expression analysis of all members of a gene family encoding cell-wall enzymes allowed us to predict cis-regulatory regions involved in cell-wall construction in specific organs of Arabidopsis. *Plant and Cell Physiology* **42**:1025–1033.

Yoshida T, Nishimura N, Kitahata N, Kuromori T, Ito T, Asami T, Shinozaki K, Hirayama T. 2006. ABA-hypersensitive germinability deficiency in Arabidopsis eto1 encodes a protein phosphatase 2C (AtPP2CA) that strongly regulates abscisic acid signaling during germination among Arabidopsis protein phosphatase 2C. *Plant Physiology* **140**:115–126.

Zhou MQ, Chen S, Wu LH, Tang KX, Lin J. 2011. CBF-dependent signaling pathway: a key responder to low temperature stress in plants. *Critical Reviews in Biotechnology* **31**:186–192.