Microarray data analysis of space grown *Arabidopsis* leaves for genes important in vascular patterning.

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Venation patterning in leaves is a major determinant of photosynthesis efficiency because of its dependency on vascular transport of photoassimilates, water, and minerals. *Arabidopsis thaliana* grown in microgravity show delayed growth and leaf maturation. Gene expression data from the roots, hypocotyl, and leaves of *A. thaliana* grown during spaceflight vs. ground control analyzed by Affymetrix microarray are available through NASA's GeneLab (GLDS-7). We analyzed the data for differential expression of genes in leaves resulting from the effects of spaceflight on vascular patterning. Two genes were found by preliminary analysis to be upregulated during spaceflight that may be related to vascular formation. The genes are responsible for coding an ARGOS like protein (potentially affecting cell elongation in the leaves), and an F-box/kelch-repeat protein (possibly contributing to protoxylem specification). Further analysis that will focus on raw data quality assessment and a moderated t-test may further confirm upregulation of the two genes and/or identify other gene candidates. Plants defective in these genes will then be assessed for phenotype by the mapping and quantification of leaf vascular patterning by NASA's VESsel GENeration (VESGEN) software to model specific vascular differences of plants grown in spaceflight.

Research supported by a NASA Ames Center Innovation Fund Award.

American Society for Gravitational and Space Research
2016 Annual Meeting, Cleveland, OH
26-29th October, 2016

Space Biosciences
NASA AMES RESEARCH CENTER

National Aeronautics and Space Administration

Analysis of Space Grown *Arabidopsis* with Microarray Data from GeneLab: Identification of Genes Important in Vascular Patterning

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INTRODUCTION

Leaves are the major photosynthetic organs of plants. Venation patterning in leaves is a major determinant of photosynthetic efficiency because of the dependence of photosynthesis on vascular transport of photoassimilates, water, and minerals. Mapping the effects of spaceflight on leaf vasculature may therefore be useful for atmospheric regeneration and crop production in future exploration and colonization of deep space.[1] Vascular transport, if hindered, could be a limiting factor in the import and export of precursors and products of photosynthesis, cellular respiration, and other processes affecting overall plant development. Using NASA's GeneLab data repository,[2] we analyzed transcriptomic data from *Arabidopsis thaliana* from spaceflight experiments[3] to discover the differential expression (DE) of genes linked to leaf venation remodeling. Our purpose is to identify genes useful for generating mutant phenocopies of spaceflight vascular patterns by ground-based studies. The efficiency of vascular patterning will then be analyzed by NASA's VESsel GENeration Analysis (VESGEN) software (patents pending). We further demonstrate the utility of NASA's GeneLab to support successful open source science.

METHODS

Differential Expression (DE) Analyses. The dataset, GLDS-7, downloaded from NASA's GeneLab contains microarray data from Paul *et al.*[2] in which transcriptiomic results are reported for three tissues: Leaf, Hypocotyl, and Root. Five replicates were obtained from both the spaceflight and ground environments, resulting in 30 microarray chips in total. A two tailed *t*-test was performed on the mean values for expression of spaceflight vs ground tissue with p-value < 0.01. The package "affy" in Bioconductor for R was used for normalization. The "expresso" parameters were as follows: normalization = "qspline", bgcorrect = "rma", pmcorrect = "pmonly", summary = "medianpolish".

Quality Control (QC). The "oligo" package in Bioconductor was used for data preprocessing. Normalized Unscaled Standard Error (NUSE, **Figure 1**) and Relative Log Expression (RLE, **Figure 2**) plots were used to test probe-set homogeneity. In order to determine if the lower quality probe-sets should be removed, a fully annotated differential expression analysis was performed on the following sub-sets: GLDS-7 5v5 [8410 – 8414 : 8415 – 8419], 5v4[8410 – 8414 : 8416 – 8419], and 4v4[8411 – 8414 : 8416 – 8419].

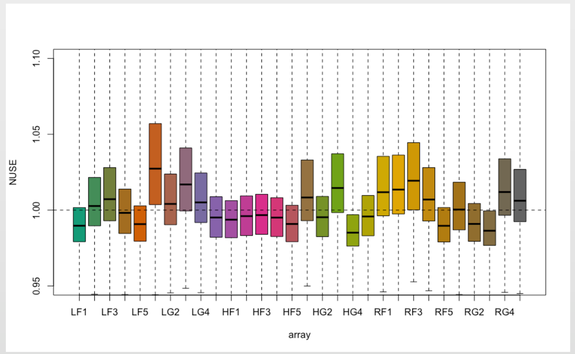

Figure 1 Normalized Unscaled Standard Error (NUSE)
Plot of all 30 microarrays. The x-axis displays each of the replicates (L, leaf; H, hypocotyl; R, root) (F, flight; G, ground) (#, replicate). The y-axis is the ratio of the [standard error estimate of the fit] : [standardized standard error estimates of the fit]. (standardize = the collective median of all of the arrays are set to 1)

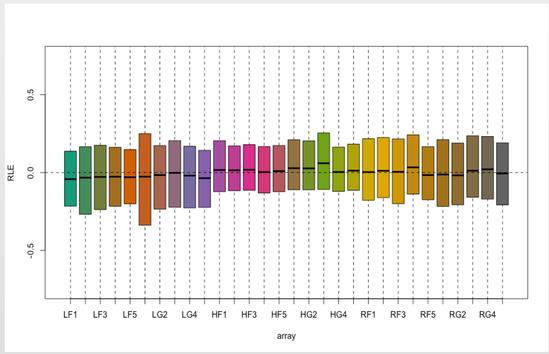

Figure 2 Relative Log Expression (RLE)
Plot of all 30 microarrays. The x-axis and naming convention is the same as in Figure 1. The y-axis is the ratio [expression of a probe-set] : [median expression of probe-set across all arrays].

RESULTS

By QC (shown in **Figures 1 & 2**), all probe-sets were within acceptable ranges of homogeneity. RNA Degradation analysis revealed that all 30 probe-sets had derivatives of similar magnitudes, demonstrating that the chips were of good quality. Analysis of the transcriptomic data identified 22 significantly DE genes, of which seven (colored in **Table 1**) have potential relations to plant vasculature. The two differentially expressed gene clusters, **K**ISS **M**E **D**EADLY [KMD] coding F-box genes (orange in **Table 1**) and **N**AM, **A**TAF1/2, and **C**UC2 [NAC] related genes (green in **Table 1** and **Figure 4**), together with their respective functions, suggest that there may be phenotypic changes in leaf venation (specifically xylem) resulting from development in a spaceflight environment.

Table 1 Genes of Interest Gene ID is the accession number used in The *Arabidopsis* Information Resource (TAIR). Fold change is a $\log_2$ fold change of spaceflight expression levels compared to ground grown expression levels. Colored genes have links to plant vasculature. Orange genes are KMD coding F-box genes, green genes are NAC related genes.

Gene ID	Fold Change	Description	Gene ID	Fold Change	Description
AT2G31945	1.863	Unknown	AT1G10340	1.224	Ankyrin repeat
AT2G20670	1.718	Unknown	AT3G07350	1.222	Unknown
AT5G14730	1.602	Unknown	AT2G27830	1.219	Unknown
AT3G15500	1.563	NAC 55	AT1G13260	1.209	AP2/ERF/B3 TF RAV1
AT1G25400	1.550	Unknown	AT1G23390	1.199	F-box/kelch-repeat
AT1G15670	1.504	F-box/kelch-repeat	AT3G59940	1.164	F-box/kelch-repeat
AT3G12500	1.419	Basic endochitinase B	AT1G21000	1.101	PLATZ TF
AT2G44130	1.284	F-box/kelch-repeat	AT5G20250	1.079	sucrose galactosyltransferase 6
AT5G41080	1.279	GDPD2	AT1G69490	1.071	NAC TF 29
AT3G13310	1.249	Chaperone DnaJ	AT2G25200	1.051	Unknown
AT1G80440	1.226	F-box/kelch-repeat	AT1G44800	-0.715	WAT1-related protein

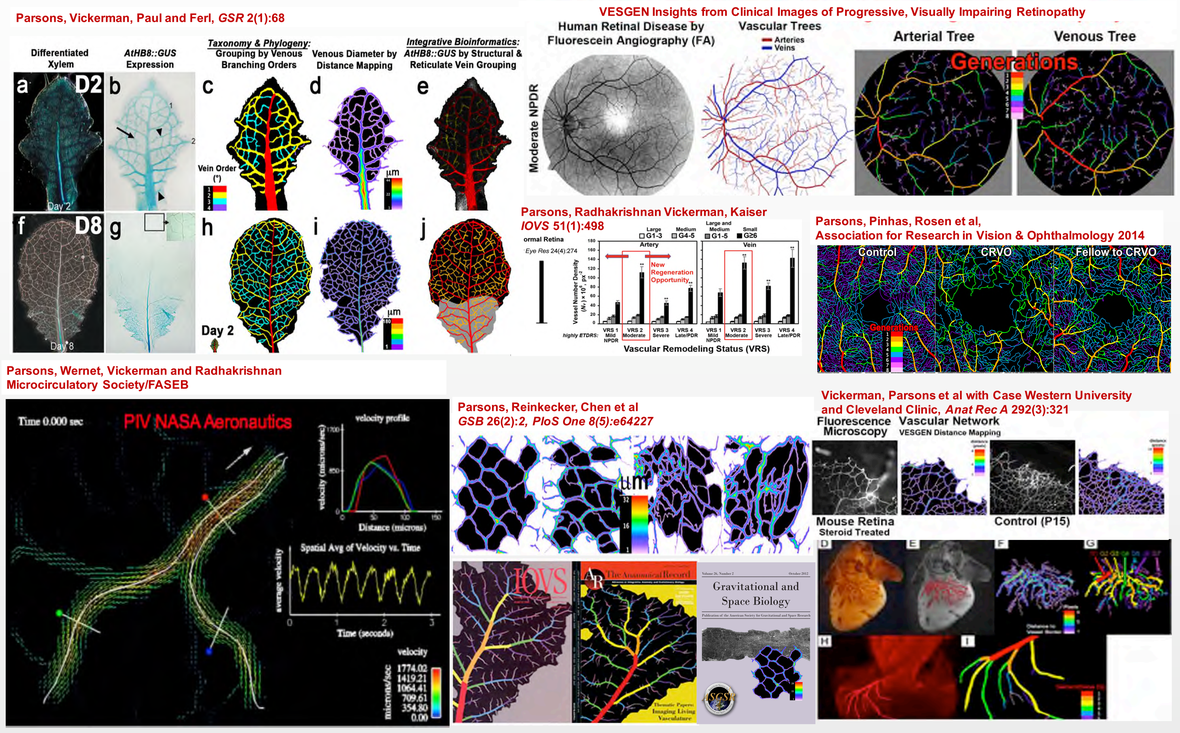

Figure 3 Analysis by NASA's VESGEN software of vascular patterning important for space biology and human space exploration
Tissues important in space biology research and human space exploration include (clockwise): Developing venation patterns in the leaves of *Arabidopsis*; the human retina, for which hypothesized vascular complications in visual impairments are being studied by NASA in images from astronauts and human bed rest subjects; mouse model of retinal vascular remodeling; progressive inflammation in the mouse GI; developing coronary vessels in mouse and avian models, and control of blood flow by vessel branching generation according to fluid mechanics. Other models analyzed by VESGEN include lymphatic development and remodeling by active stem cell recruitment.

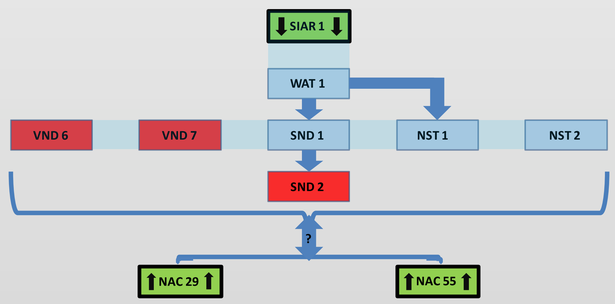

Figure 4 Theoretical Transactivation of Xylem Specifying NAC Domains
Each box represents a gene, specifically a NAC except for the **SI**LIQUES **A**RE **R**ED 1 [SIAR 1] and **W**ALLS **A**RE **T**HIN 1 [WAT 1]. Blue arrows denote activation and transactivation of other genes. The question mark shows a potential transactivation. Black arrows denote the direction of the change in expression levels during spaceflight compared to ground control. Boxed arrows pointing upwards indicate upregulation, and arrows in boxes pointing downwards, downregulation. The blue highlights connecting SIAR 1 and WAT 1, as well as the horizontal cluster of genes, denote homology between the respective clusters. The green boxes represent genes that are found to be differentially expressed in our analysis (colored green in **Table 1**). The red boxes represent NACs that are specifically related to vascular development.

CONCLUSIONS

- Two gene clusters pertaining to leaf venation were revealed in the Differential Expression Analysis: NAC (green in **Table 1**) and KMD (orange in **Table 1**)
- Expression of KMD is inversely correlated with cytokinin sensitivity. Cytokinin sensitivity is inversely correlated with protoxylem specification. This implies KMD is directly correlated to protoxylem specification
- **V**ASCULAR RELATED **N**AC **D**OMAIN [VND] 6 and 7 (red in **Figure 4**) are "xylem master switches" and may be upregulated via transactivation, a trait already observed in some NAC domain genes
- Existing SALK lines, mutated in the NAC genes (green in **Table 1**), already show xylem related phenotypic changes
- As a proof of technology experiment,[1] reticulate vein density within one leaf of *Arabidopsis thaliana* was altered when grown in spaceflight compared to ground control
- Our findings will allow VESGEN software to corroborate these results by performing analysis on ground grown mutant phenocopies of the colored genes in **Table 1**

REFERENCES

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ACKNOWLEDGMENTS

Supported by NASA Ames Center Innovation Fund Award (PPW) with USRA Student Internship to AW; NASA Space Life and Physical Sciences Program (PPW); Dr. Wyatt. We thank Colin Kruse, Ohio University and Homer Fogle, NASA GeneLab, for helpful discussions.