The Effects of Hypoxic Stress on respiration and RAP2.12p::RAP2.12:YFP in *Arabidopsis thaliana*

Samuel Moss*[1], Anne Poll*[2], Brittany Russell*[1], Caleb Fitzgerald*[3], Amanda Salvi[4], Richard Barker[4], Won-Gyu Choi[5], Sarah Swanson[4], and Simon Gilroy[4]

UW-Madison Affiliations: Departments of Biomedical Engineering[1], Industrial Engineering[2], Biological Systems Engineering[3] and Botany department[4] at the University of Wisconsin-Madison. [5]Department of Biochemistry and Molecular Biology, University of Nevada-Reno. Contributed equally*

30 second summary

- Hypoxic chambers were built to try to replicate gas accessibility effects of spaceflight using a range of oxygen concentrations.
- Col-0 Arabidopsis expressing RAP2.12p::RAP2.12:YFP were grown in the chambers set at 6% to ambient oxygen concentrations and confocal imaging revealed localization of RAP2.12 to the nucleus under hypoxic conditions.
- As expected, respiration decreased under hypoxic stress, however, stomatal conductance was increased.

Introduction

Spaceflight is a stressful environment that can affect plant growth via a range of different mechanism (reviewed in 1). Analysis of the transcriptomics of spaceflight grown Arabidopsis (2) implied that plants are experiencing both hypoxic and oxidative stress during spaceflight. Combing these data with meta-analysis of other previous plant spaceflight experiments revealed that transcriptional alterations to the expression of mitochondria- and chloroplast-related genes is common across multiple experiments indicating that localized hypoxia may be a common stress associated with the microgravity environment on board the International Space Station. To investigate this possibility we developed a series of automatically controlled hypoxic chambers and used photosynthetic efficiency, stomatal aperture qPCR and subcellular localization of the ethylene & hypoxic responsive RAP2.12:YFP protein (3).

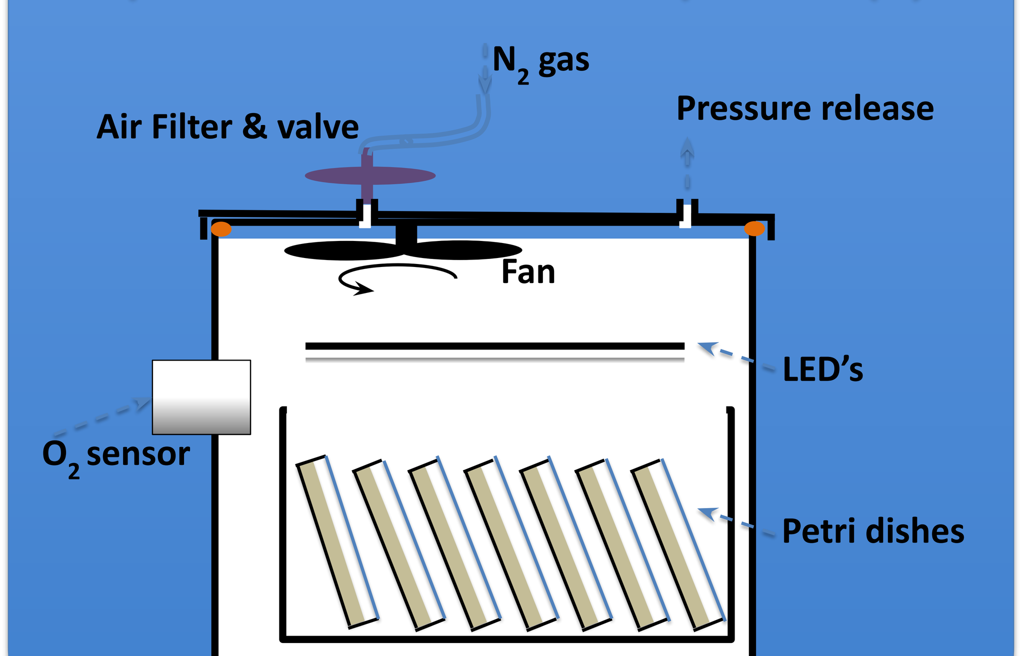

Figure 1: Diagram of hypoxia chambers

Phenotypic Methods & Results

Seeds were sown on media containing 1% Phytagel, 0.3% sucrose and ½ strength LS. Seeds were stratified in darkness for 3 days at $4^{O}C$.

1. Samples prepared for microscopy were put into the hypoxic chambers and grown for 16 days until imaging.
2. Samples prepared for stomatal conductance, respiration and photosynthetic efficiency analysis were grown at ambient O_2 for 2 weeks before being transferred to the hypoxic chambers for 48 hours and subsequently measured with a Licor LI-6400.
3. Samples prepared for qPCR were grown in the hypoxic chambers for 8 days before RNA extraction.

1. Confocal analysis

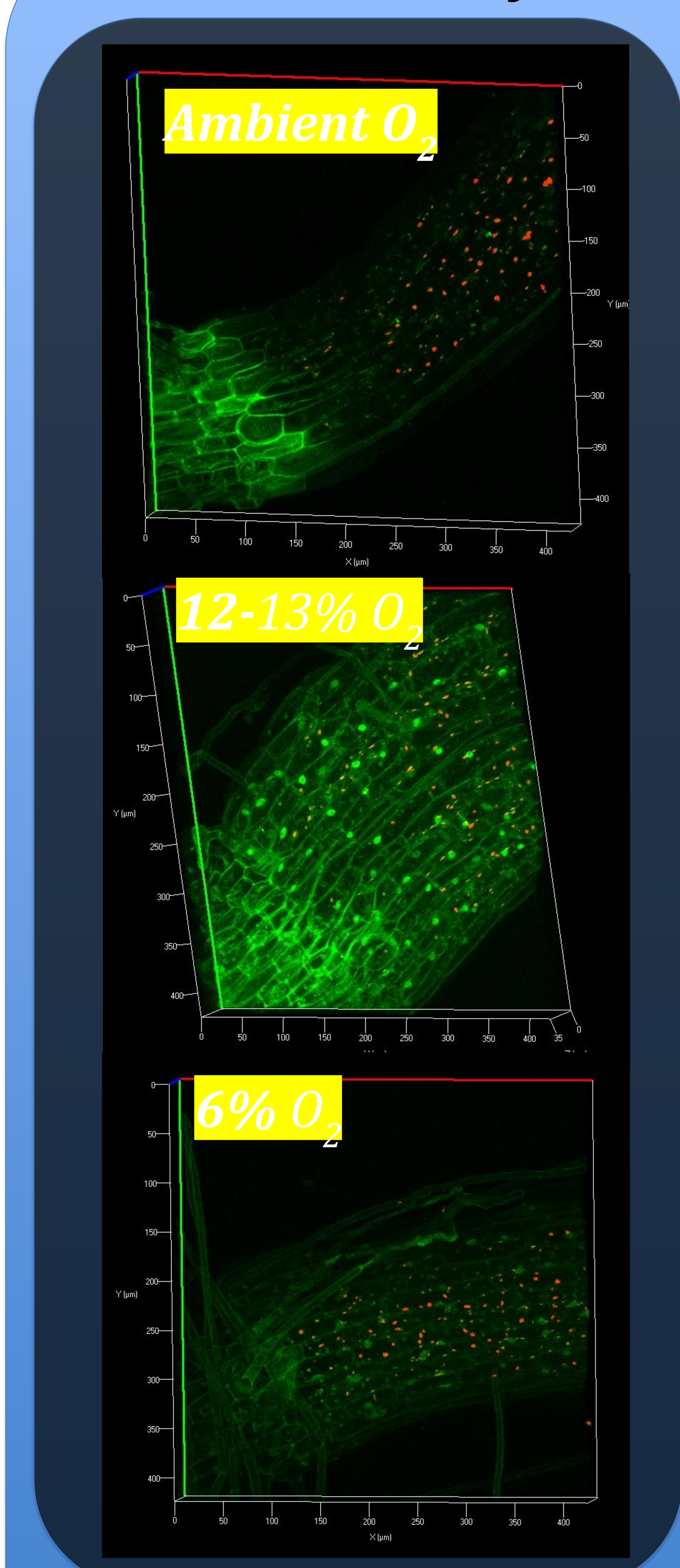

Figure 2: Confocal microscope images showing RAP2.12:YFP localization in the hypocotyl during hypoxic stress.

2. Gas exchange analysis

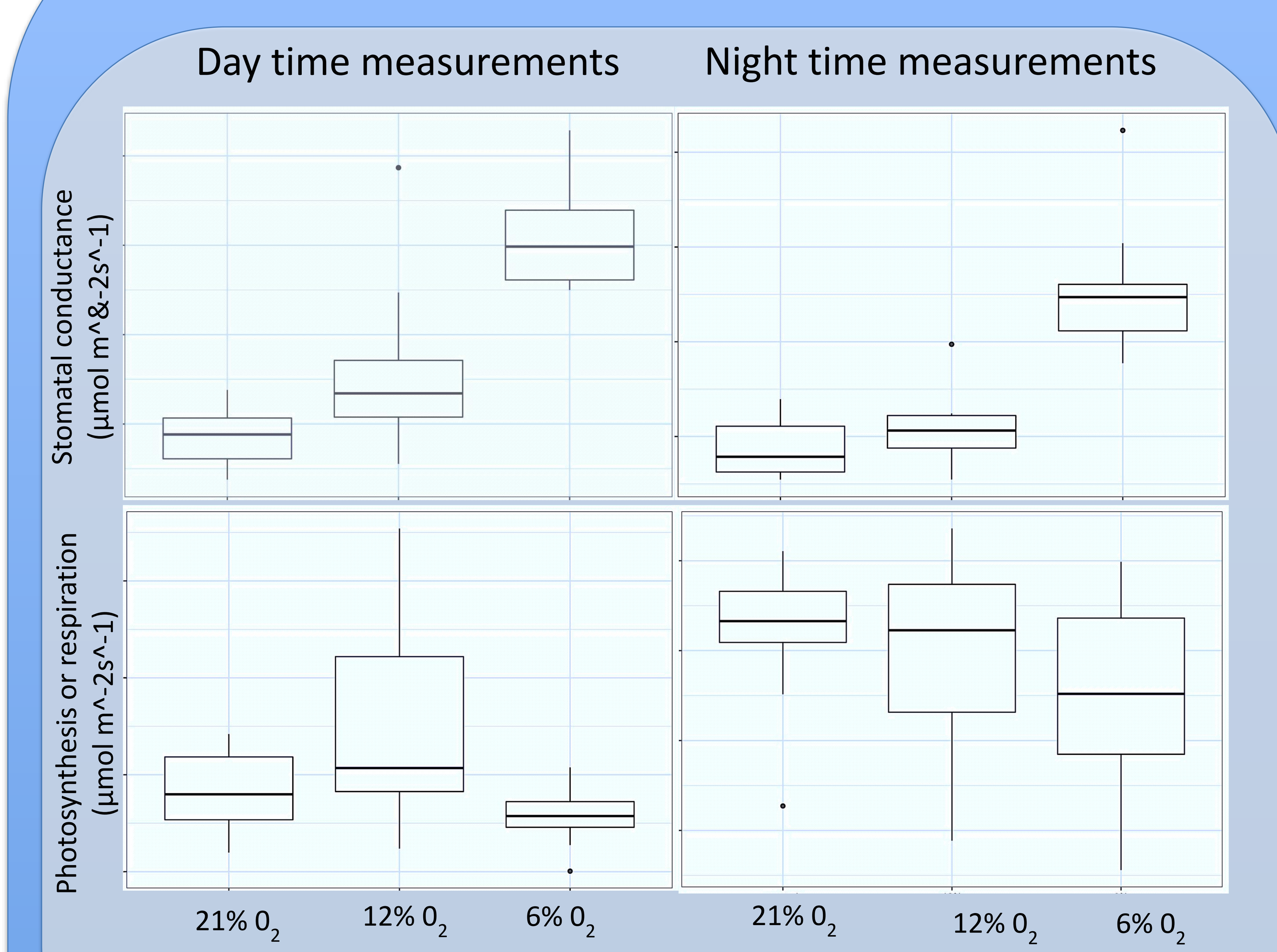

Figure 3: Stomatal conductance and photosynthesis / respiration for WT Col-0 during hypoxic stress.

3. qPCR analysis

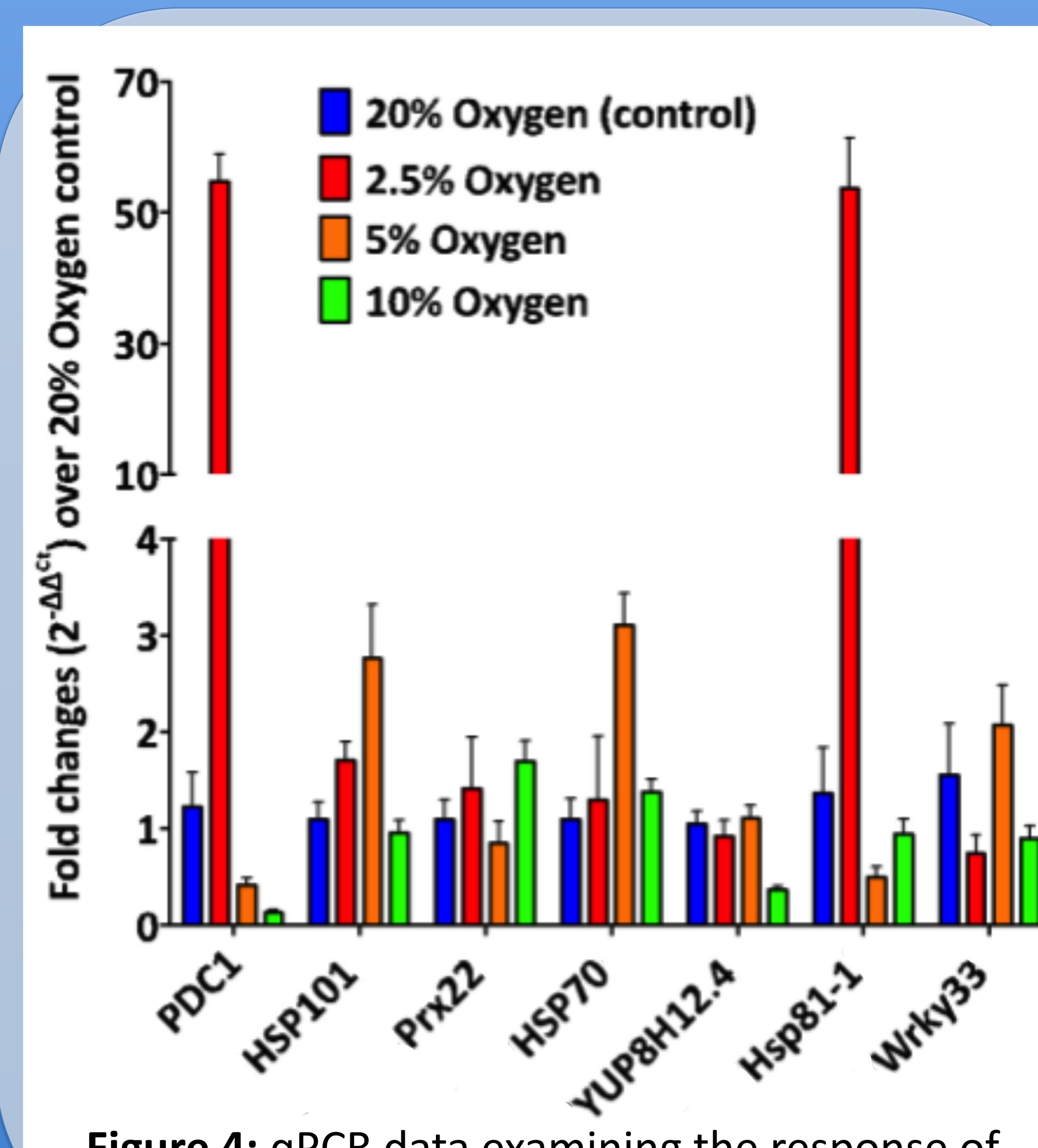

Figure 4: qPCR data examining the response of spaceflight responsive genes to hypoxia

Conclusions

- The hypoxic chambers were able to trigger RAP2.12:YFP accumulation in the nucleus.
- Plants transferred to low oxygen environment open their stomata and this response is more severe in lower concentrations of oxygen.
- Increasing the stomatal conductance in response to hypoxia likely helps the plant maintain photosynthesis and respiration rates.
- qPCR identified hypoxia responsive transcripts. Cross-referencing to the spaceflight transcriptome, the responses of *HSP101*, *HSP70*, *HSP81-1*, *YUP8H12.4* and *WRKY33* at 2.5-5% O_2 all mirror spaceflight-induced changes, although the classic hypoxic marker *PDC1* does not.

References

1. Life in space isn't easy, even if you're green (2017). Barker and Gilroy. The Biochemist. Dec 2017.
2. Ecotypic variation in the Arabidopsis transcriptome in response to spaceflight reveals a role for heat shock proteins, hypoxia and oxidative Stress (2018). Choi, Barker, Kim, Swanson and Gilroy (in review).
3. The stability and nuclear localization of the transcriptionfactor RAP2.12 are dynamically regulated by oxygen concentration (2015). Kosmacz et al., Plant, Cell and Cnvironment. 38:1094-1103.