Potential Role of AHA2 Protein in Plant Gravity Response

Victoria A. Swiler[1], Calvin Coffin[1,2], Nicolás Barr[1], and Sarah E. Wyatt[1,2]

[1]Department of Environmental and Plant Biology, [2]Interdisciplinary Molecular and Cellular Biology Program
Ohio University, Athens, Ohio 45701

Introduction

- Despite the necessity of gravity response for plant survival, many of the steps in **gravity signal transduction** are not well understood.

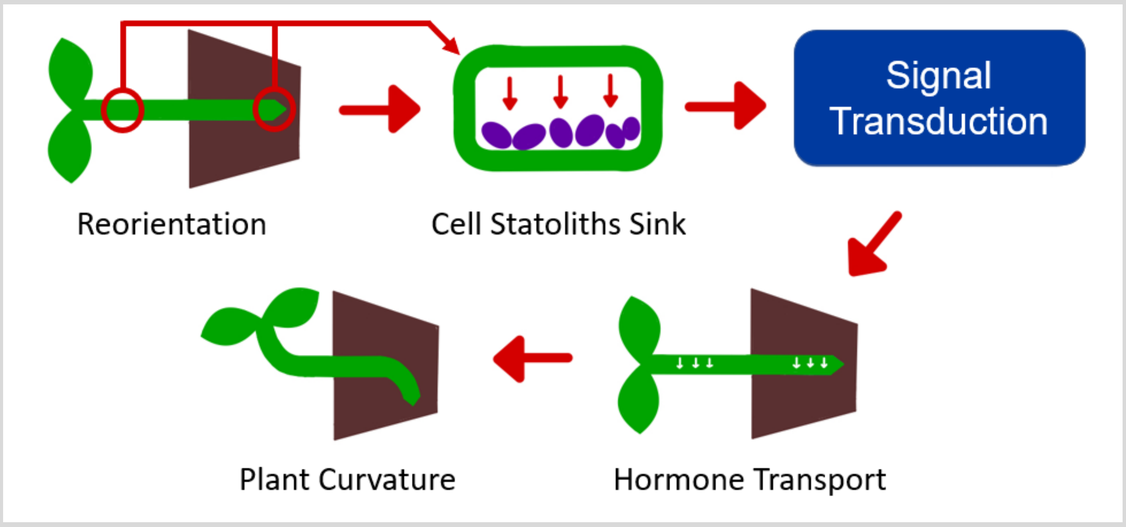
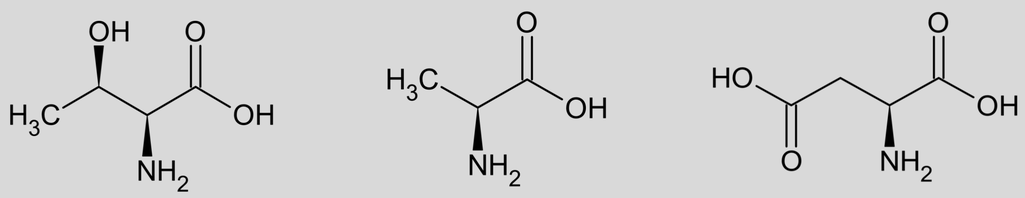


Figure 1: Plant gravitropism. Plants respond to gravity through a series of steps known as gravitropism.

- The phosphorylation (activation) of the **AHA2 protein** is suspected to play a role in gravity signaling in *Arabidopsis thaliana* based on an ISS experiment where AHA2 proteins were less phosphorylated in spaceflight compared to ground controls[2].
- AHA2 stands for Arabidopsis plasma membrane proton ATPase 2 and acidifies the plant cell wall.
- **<u>Hypothesis</u>:** The phosphorylation of the AHA2 protein links hormone movement and differential growth in gravitropism.



Threonine (T) **Native** | Alanine (A) **Constitutively Inactive** | Aspartic Acid (D) **Constitutively Active**

Figure 2: Phosphorylation site amino acids. Changing the phosphorylation site from threonine to alanine prevents phosphorylation, keeping AHA2 inactive. Changing it to aspartic acid biomimics phosphorylation, keeping it active.

- 7 *AHA2* gene constructs with two altered phosphorylation sites will be inserted into Arabidopsis:
  - T947A
  - T942A
  - T942-947A
  - Unaltered (recovery)
  - T947D
  - T942D
  - T942-947D
- By phenotyping Arabidopsis with altered AHA2 phosphorylation sites, the role of AHA2 phosphorylation in signal transduction will be determined.
- **A better understanding of plant gravitropism will pave the way toward developing spaceflight-tolerant agricultural plants for long-term space habitation!**

Methods

Develop *AHA2* gene with altered phosphorylation sites → Transform genes into Arabidopsis via *Agrobacterium* → Phenotype mutants for altered gravity response

Figure 3: **Steps in determining importance of AHA2 in gravity response.**

Phenotyping

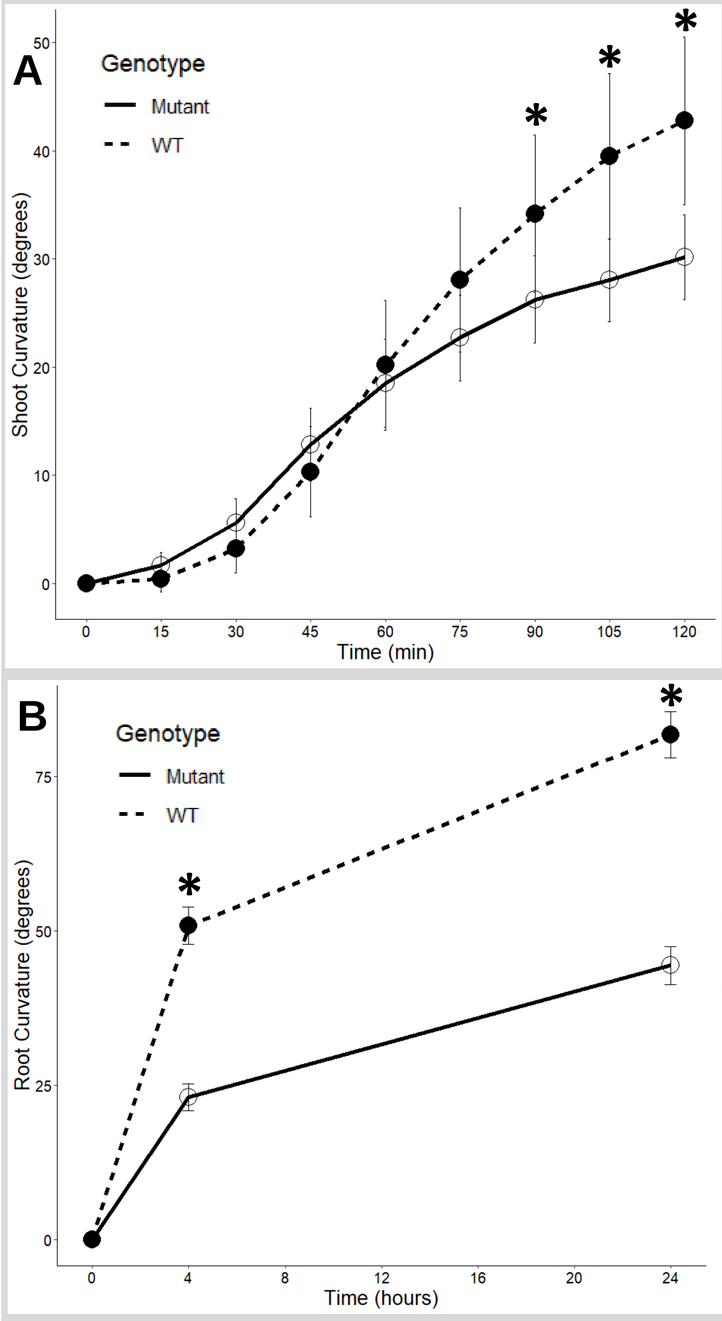


Figure 4: ***aha2* knockout mutants have reduced stem and root curvature**.
A) Using a gravity persistent signaling (GPS) experiment, it was determined that *aha2* knockout mutants have reduced stem curvature compared to wild-type (WT) Arabidopsis at 90, 105, and 120 min.
B) Using a root curvature experiment, it was determined that *aha2* knockout seedlings have reduced root curvature compared to WT seedlings. Therefore, AHA2 is likely involved in Arabidopsis gravity signal transduction.

***** = $p<0.05$, ANOVA test

Future Work

- The remaining gene constructs with altered phosphorylation sites **(Figure 2)** will be created and transformed into Arabidopsis *aha2* knockouts.
- Transformed seeds will be phenotyped for altered gravity response to determine if AHA2 phosphorylation is involved in gravity signal transduction.
- **Become one step closer to genetically engineering plants for survival in long-term space habitation!**

Figure 5: ***A. thaliana*[1].**

References

[1]Arabidopsis Biological Resource Center [ABRC]. (n.d.). *An Introduction to Arabidopsis thaliana.* https://abrc.osu.edu/educators/about-arabidopsis. [2]Kruse, C. P. S. et al. (2020). Spaceflight induces novel regulatory responses in Arabidopsis seedling as revealed by combined proteomic and transcriptomic analyses. *BMC Plant Biology, 20,* https://doi.org/10.1186/ s12870-020-02392-6

Acknowledgements

Partial funding was provided by the Ohio University Department of Arts and Sciences, the Ohio Space Grant Consortium, and the Ohio University Research Incentive Fund to S.E.W.