The effect of mechanical stimulation on crown root development in the model grass *Brachypodium distachyon*

UMassAmherst
College of Natural Sciences
Center for Agriculture, Food, and the Environment

Eleah Flockhart, Greg Gregory, Emil Mah, Ian McCahill, Kira Gardner, Joshua H. Coomey, Samuel P. Hazen

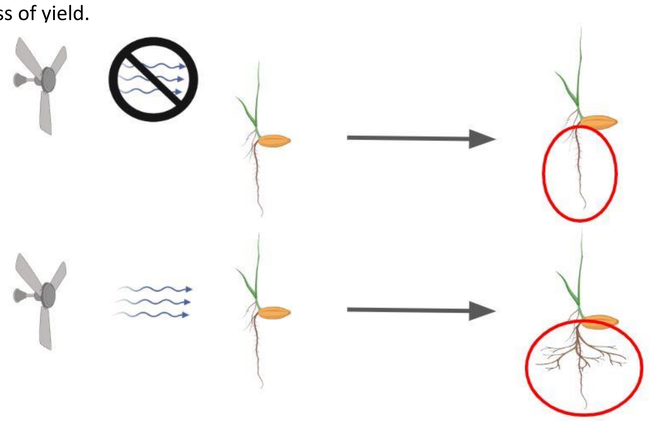
United States Department of Agriculture
National Institute of Food and Agriculture

Abstract

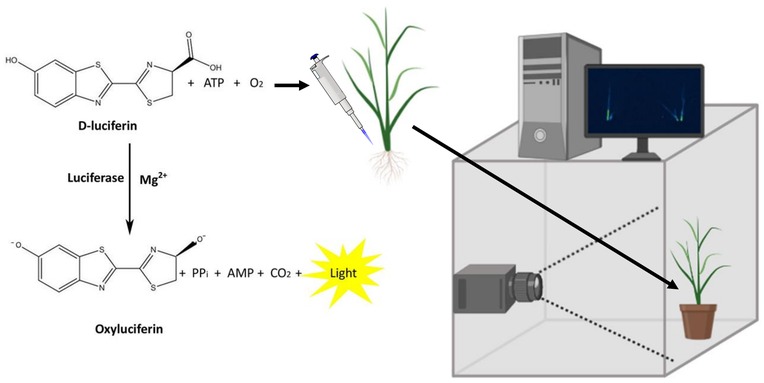
Cereal crops are the most prominent species in agriculture worldwide. However, under the weight of impressive seed production, plants can fall over in the field, known as lodging, by bending of the stem near the ground which results in severe loss of yield. The shoot-borne roots at the base of the stem can provide the necessary stability to avoid lodging. The goal of the study was to investigate the development of shoot-borne root systems in the model grass *Brachypodium distachyon*, in response to mechanical stimuli. Previous studies have been unable to observe root growth in soil throughout development, due to the opacity of the soil. We visualized below ground roots using a bioluminescent reporter system where plants constitutively express firefly luciferase with a *ZmUBI1::LUC* transgene. Luciferase catalyzes a reaction with the substrate luciferin, resulting in light emission. By imaging with a highly sensitive charged-coupled device camera we visualized this bioluminescence. Plants were grown at a 17° angle in a mixture of large and small porous kiln fired clay granules in 3D printed rhizotrons with glass sides so the roots grow along glass to facilitate visualization. The "thigmomamtic" robot moved a bar across the plants every 90 minutes to provide mechanical stimulation. The touched group of plants reached a much lower height than the untouched group, indicating the brushing affected the entire plant. The growth medium deterred normal root growth. A growth medium composed of a mixture of large clay granules and coco coir resulted in normal root growth, and thus will be used in future experiments on the effects of mechanical stimuli on shoot-borne root growth.

Introduction

Plants can sense and respond to mechanical stimuli, such as brushing, touching, bending, or wind[1]. Mechanical stimuli on the above-ground portion of the plant can affect the below-ground portion of the plant. Shoot borne roots can allow the plant to adapt to environmental stress. *B. distachyon* plants exposed to constant wind will grow shoot borne roots that provide more stability in strong winds[2]. The two main types of shoot borne, or crown, roots in *B. distachyon* are coleoptile nodal roots and the leaf nodal roots[3]. The effect of mechanical stimuli on the crown roots of cereal crops is important in agriculture, particularly in regard to lodging which occurs when environmental stress, such wind or driving rain causes the stem to bend. With the stem bent, the plants lie along the ground, leading to severe loss of yield.



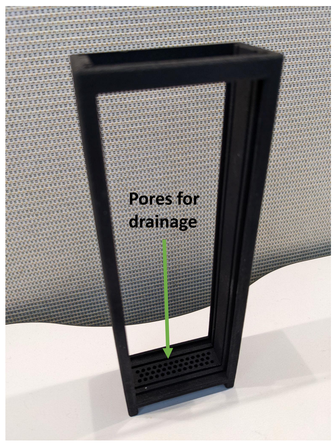
Methods: Bioluminescent Imaging

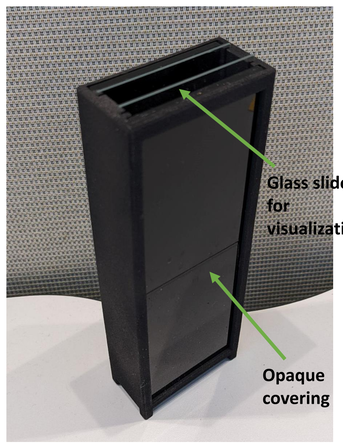


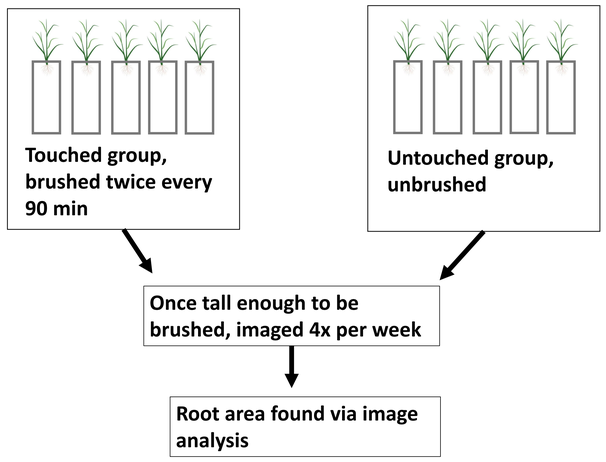
Luciferin is added to the base of the plant, and the resulting bioluminescence can be imaged with a CCD camera.

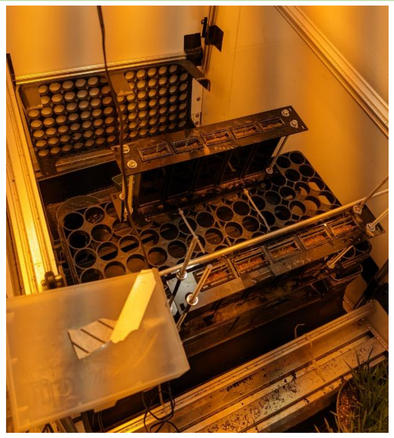
Methods: 3D Printed Rhizotrons and the Thigmomatic

Rhizotrons









The thigmomatic plant brushing robot, brushed twice every 90 minutes

Results: Touched plants were shorter

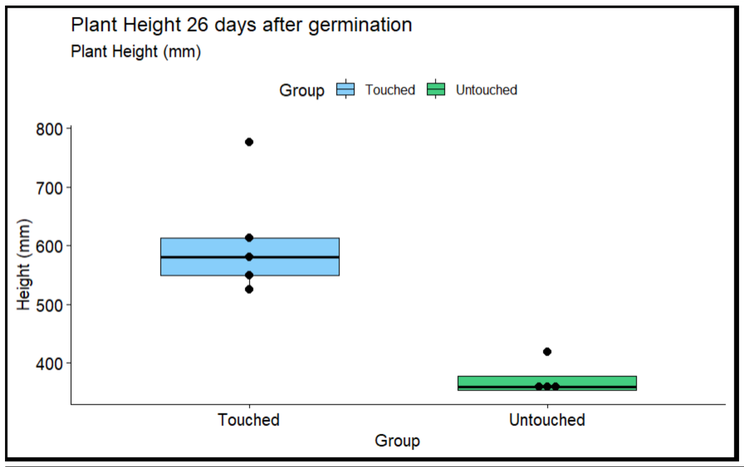
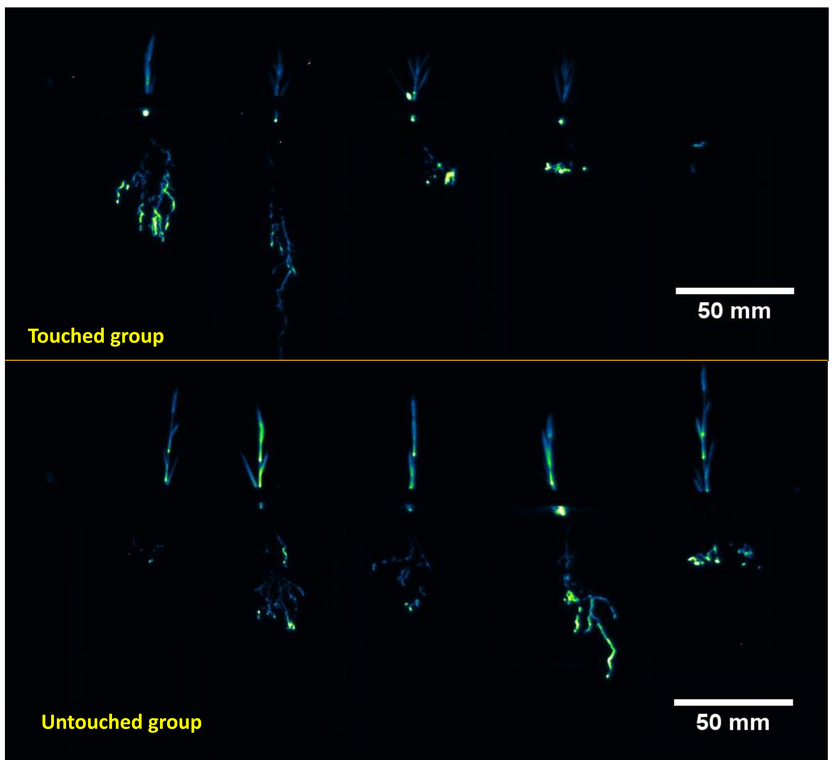
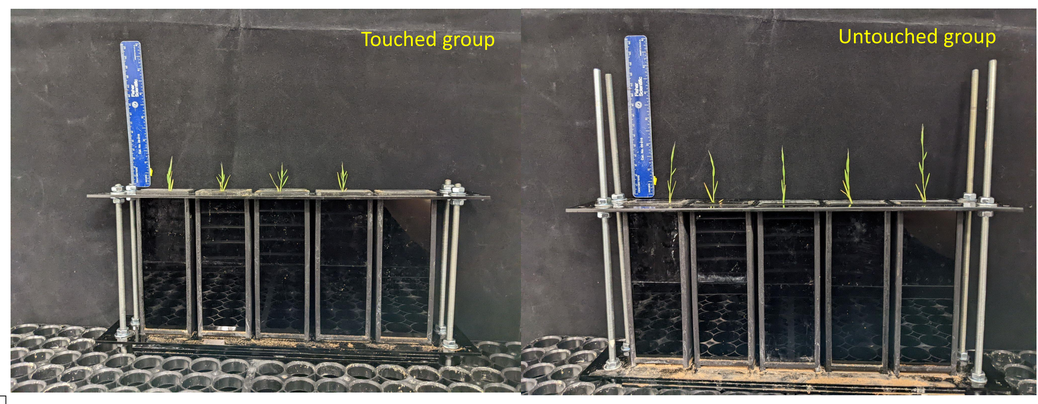


Figure 1 (above): Plant heights in mm of touched and untouched groups 26 days after germination.
Figure 2 (below): Bioluminescence images from the CCD camera of touched and untouched groups.

Bioluminescence Images 26 days after germination

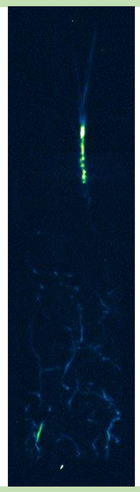


Height Comparison at 26 days after planting



Conclusions/Future Direction

A *BdCESA8*$_{pro}$*::LUC B. distachyon* plant grown in a mixture of 98 percent large porous kiln fired clay granules and 2 percent coco coir, which does not show stunted root growth.



- **Brushing twice every 90 minutes is sufficient to influence the growth of *B. distachyon*.**
- **A growth medium composed of a mixture of large and small porous kiln fired clay granules is unusable for future experiments on *B. distachyon* roots in rhizotrons.**
- **Future experiments will be performed in a growth medium of a mixture of large porous kiln fired clay granules and 2 percent coco coir.**
- **Future experiments will examine the effect of mechanical stimuli on the expression of genes in shoot-borne roots in *B. distachyon*.**

Acknowledgements

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References

[1]Ghosh, R., Barbacci, A., Leblanc-Fournier, N. Mechanostimulation: a promising alternative for sustainable agriculture practices, *Journal of Experimental Botany*, Volume 72, Issue 8, 2 April 2021, Pages 2877–2888, https://doi.org/10.1093/jxb/erab036
[2]Nam, B.E. et al. "Auxin mediates the touch-induced mechanical stimulation of adventitious root formation under windy conditions in Brachypodium distachyon." *BMC plant biology* vol. 20,1 335. 16 Jul. 2020, doi:10.1186/s12870-020-02544-8
[3]Chochois, V., Vogel, J.P., Rebetzke, G.J., Watt, M. Variation in Adult Plant Phenotypes and Partitioning among Seed and Stem-Borne Roots across *Brachypodium distachyon* Accessions to Exploit in Breeding Cereals for Well-Watered and Drought Environments, *Plant Physiology*, Volume 168, Issue 3, July 2015, Pages 953–967, https://doi.org/10.1104/pp.15.00095