# Enhancing Plant Growth in Space through Mechanical Stimulation

Ainsley Salisbury, Jessica Fernandez, Simon Gilroy
*Department of Botany, University of Wisconsin-Madison, WI 53706*

## Abstract

In space, plants are exposed to a different environment than they are on Earth, including microgravity reducing mechanical loads and altering the spectrum of mechanical signals the plant experiences. By characterizing the plant molecular sensing system through the response to mechanical touch, we can understand stress response in plants on Earth, define how this may be altered in spaceflight, and potentially increase plant durability in both environments. Calcium signaling is also known to be an important pathway that initiates these touch-triggered responses. To evaluate the role of calcium signaling in the plants' stress response, plants were mechanically stimulated in a controlled and reproducible way using the Automated Botanical Contact Device (ABCD). To better understand the link between calcium signals and stress response, three mutants (*cpk1, cpk5*, and *glr3.3glr3.6*) were evaluated and compared to the wild type *Col-0*. We found that mutants with disturbed calcium signaling had altered growth phenotype when mechanically stimulated for 5 days. By understanding the impact of touch stress on root system morphology, we can develop strategies for improving plant growth both on Earth and in spaceflight conditions.

## Introduction

On Earth, plants are inevitably exposed to the environment. This can include both biotic stimuli, like pathogen and herbivore attacks, and abiotic stimuli, like unpredictable weather. Such mechanical stimulation can lead to directional growth to or from the touch stimulus or a more general change in growth habit, often leading to reduced vegetative growth and increased production of strengthening tissues, a response known as thigmomorphogenesis (Börnke and Rocksch 2018). In space, some of these environmental stressors are comparable to those seen on Earth, such as pathogen attack or flooding, but some are very different, as in reduced gravity levels or increased radiation. The response to gravity in plant growth is a branch of thigmomorphogenesis that involves the restructuring of a plant in response to its own weight (Hoson, 2014). In spaceflight, the microgravity environment means that the mechanical signal from gravity that normally triggers these responses is absent, and so spaceflight is thought to correspond to environments with reduced mechanical signaling in plants on Earth.
Understanding how plants respond to mechanical environments in spaceflight will be critical to

developing robust plants to help feed and support crew life. Further, defining space-related countermeasures for these plant stresses could impact food production on Earth.

Current research in the Gilroy lab shows that these mechanical signals are linked to defense, which makes it probable that plants are more susceptible to pathogens in the absence of gravity. Unlike in mammalian systems, the plant molecular sensing system for mechanical stimuli and gravity perception, as well as the downstream networks they trigger, is poorly defined. The research in this project seeks to characterize such mechanical signaling in the model plant *Arabidopsis thaliana*, providing key information about internal plant responses and their molecular components with potential application to both spaceflight and terrestrial agriculture.

**Background**

In order to evaluate mechanical response to touch, a way to stimulate the plants is needed. The Automated Botanical Contact Device (ABCD) is crucial for keeping experiments controlled–allowing reproducible, recurring stimulation (Figure 1). This device uses a computerized platform that drags a plastic sheet intermittently across plants as they grow, thus imitating the potential stress of touch in the wild. The ABCD is also able to track morphological changes as the plants develop using automated computer vision (Fitzgerald 2022).

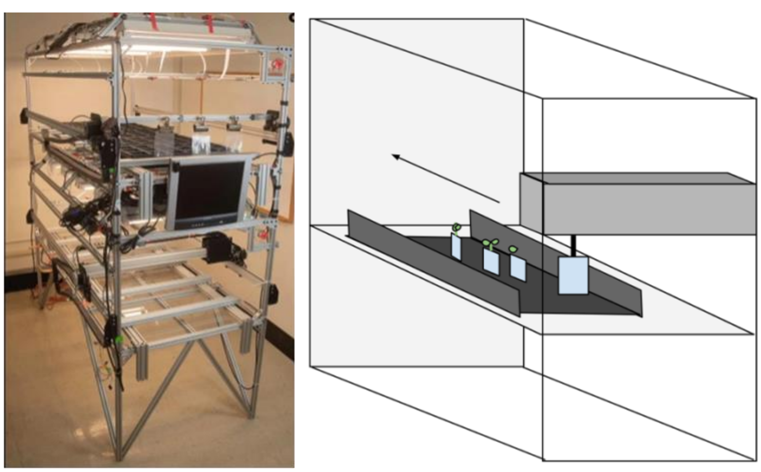


Figure 1. The Automated Botanical Contact Device (left) is used to create controllable and reproducible contact to *Arabidopsis thaliana*. Arabidopsis is grown in modified Petri plates (see Figure 3 below), which are lined up on the device (pictured right) next to untouched controls and allowed to grow for 5 days. Reproduced from Fitzgerald et al (2022) with permission.

The aerial parts of plants grown in soil are known to display a significantly smaller rosette phenotype in response to touch (Fitzgerald 2022; Figure 2). Although differences in the foliage are readily observed in these kinds of experiments, potential effects on the root system are not easily detectable since the roots are hidden in soil. This experiment sought to overcome this limitation and to understand how roots respond–on the physical and molecular levels–to their leaves being touched.

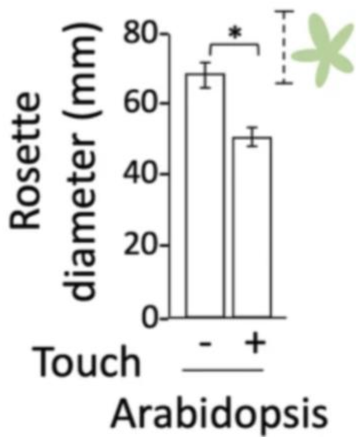



Figure 2. Rosette diameter in response to touch. The diameter in touched plants (+) was significantly decreased when compared to plants that were not touched Reproduced from Fitzgerald et al (2022) with permission.

Typically, root studies with Arabidopsis are done on Petri dishes containing a transparent growth media specific to plants, which allows ease of visualization and analysis of roots. The media consists of a transparent gel (~1% (w/v) Phytagel) in an aqueous solution with mineral nutrients to support the plant's growth (typically Murashige and Skoog or Linsmaier and Skoog salts). Throughout the experiment, both the root and shoot system of the plant remain within the sealed Petri dish, developing on the surface of the gel. To adapt this approach for use in the ABCD, we grew plants on upright Petri dishes filled with media (Figure 3). A small hole drilled in the top of the system allows the seed to be placed in the top of the gel so the roots grow into the gel and the leaves grow out in the open—available for stimulation by the ABCD. With this method, root development can be followed in situ without disturbing the plant. Examples of observable root phenotypes include primary root length, number of lateral roots, and number of root hairs.

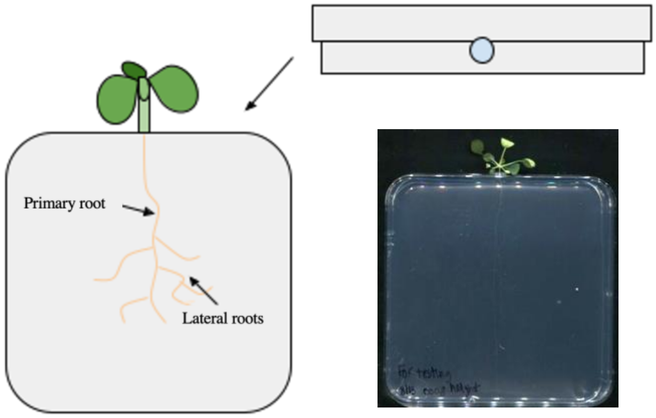
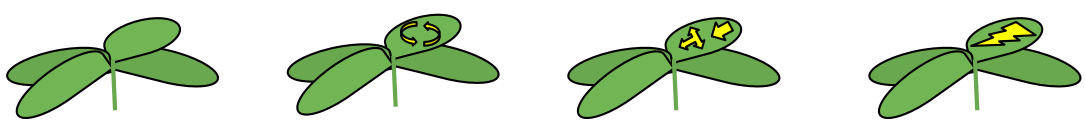



Figure 3. Depiction of *Arabidopsis thaliana* growing in an upright plate. Primary root length and lateral roots are visible through the transparent media. Growth can be monitored during development on the ABCD without disturbing the plant.

There is no current information about how touch affects root development, but this system can characterize these events. The ABCD allows us to screen for mutants that are predicted to be either insensitive or hypersensitive to mechanical stimulation. Selection of genes to knock out and analyze (Figure 4) was drawn from models that describe systemic signaling in plants (e.g., Monshausen and Haswell 2013; Toyota and Gilroy 2013). Differences between these mutants and the wild type roots will help us understand where in the cascade of touch signaling the genes are acting.



*Col-0*: Wild type plant; Columbia Arabidopsis accession.

*cpk1*: CALCIUM PROTEIN KINASE 1. Involved in cellular homeostasis.

*cpk5*: CALCIUM PROTEIN KINASE 5. Involved in ROS-dependent cellular communication.

*glr3.3glr3.6*: GLUTAMATE RECEPTOR-LIKE proteins. Double mutant attenuates wound activated electrical signal propagation between leaves.

Figure 4: Mutants involved in this experiment with a description of the normal function that is associated with these genes.

## Methods

Holes were put in the side of 10 cm square Petri plates for seed planting. These were filled with a transparent growth media (1% w/v Phytagel, ½ strength Linsmaier and Skoog salts) and sealed off. Once solidified, *Arabidopsis thaliana* seeds were planted through the hole on the exposed media and left to grow for 10 days. The young seedlings were then set up on the ABCD for repetitive stimulation every 15 minutes for 5 days. The first round of mechanical stimulation included *Col-0* wild type and the mutant *cpk1*, and the second round included *Col-0, cpk5*, and *glr3.3glr3.6*. After 5 days, each plate was scanned and analyzed with ImageJ (Schindelin et al, 2015) to obtain measurements for number of lateral roots, primary root length, and shoot area.

## Results

Similar to what was reported in Fitzgerald et al (2022), treatment with the ABCD led to the wild type *Arabidopsis thaliana* having smaller average shoot area. Figure 5 shows that the mutants *cpk1, cpk5*, and *glr3.3glr3.6* all had no statistically significant difference in shoot area in response to touch, although the glr3.33.6 mutant was quantitatively larger to begin with.

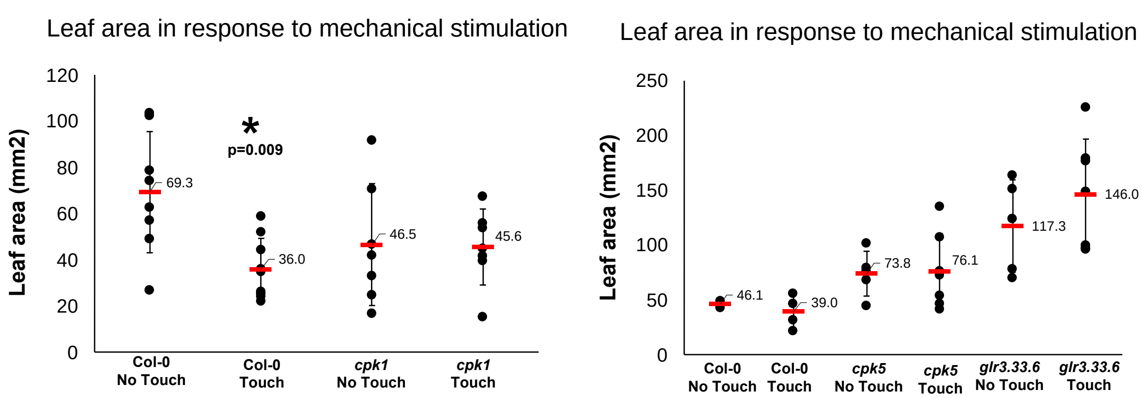



Figure 5: Graphs showing shoot area by plant, *col-0* (far left) is the only genotype with a significantly different shoot size. P-value shown with asterisk, students t-test. The mutant genotypes did not have any significant differences.

Wild type plants also had a significantly shorter primary root length when mechanically stimulated, compared to the mutant plants, which showed no significant phenotype.

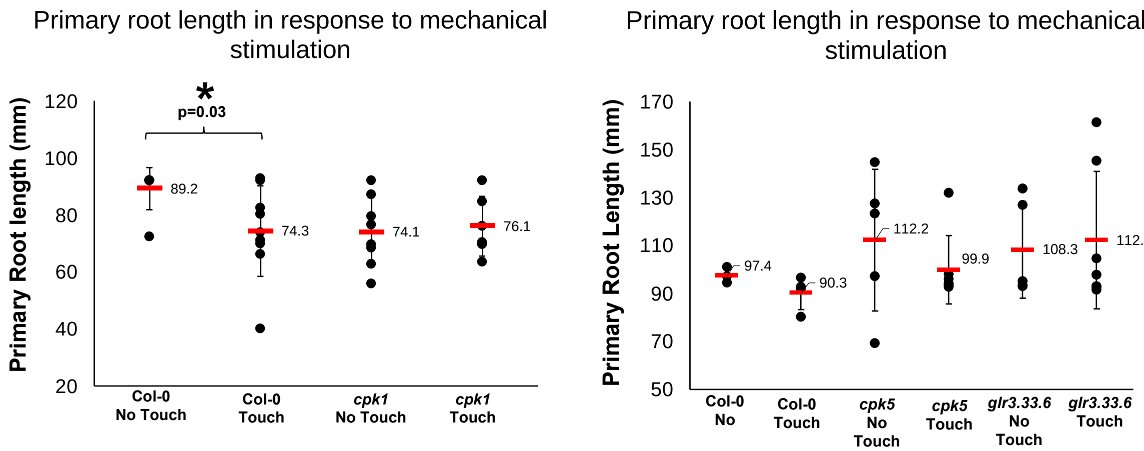



Figure 6: Graphs showing primary root length by plant, *col-0* (far left) is the only genotype with a significantly different root length. P-value shown with asterisk, students t-test. The mutant genotypes did not have any significant differences.

Finally, the wild type plants grew significantly fewer lateral roots when mechanically stimulated, whereas, again, the mutant plant roots had no observable differences in root branching.

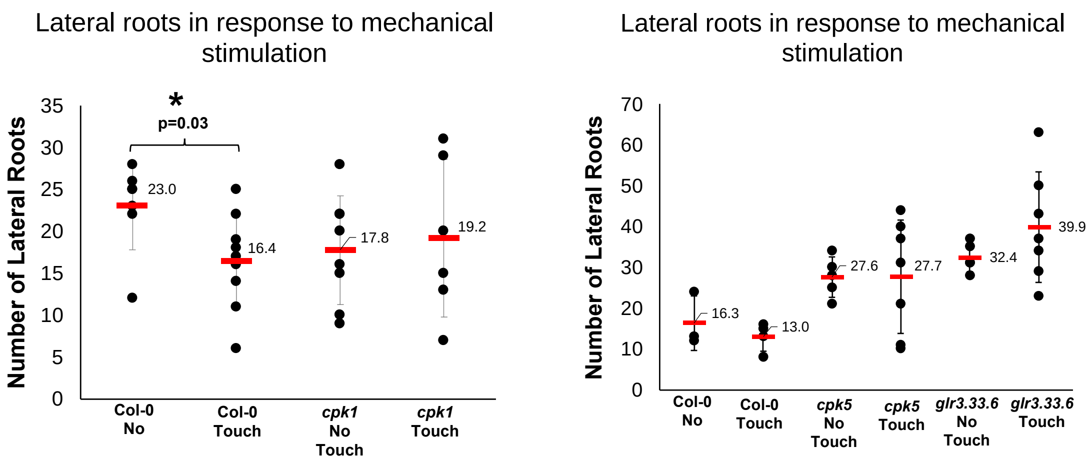



Figure 7: Graphs showing number of lateral roots per plant, *col-0* (far left) is the only genotype with a significantly different number of lateral roots. P-value shown with asterisk, students t-test. The mutant genotypes did not have any significant differences.

Intermittent touch decreases wild type (*col-0*) plant size, both in shoots and in roots; however, the *cpk1* mutant plants do not appear to have equivalent effects in the resulting touch phenotype, suggesting CPK1 may play an important role in the perception of touch response.

Preliminary data for knockouts in *cpk5* and *glr3.3glr3.6* mutants also suggest that these genes play important roles in plant touch response, although this needs further replication for definitive conclusions. With further exploration of these mutants and their associated phenotypes, the mechanical stress response from these mutants could be a key part of crop yield or growth optimization for future spaceflight experiments.

**Acknowledgements**

This project was funded by the Wisconsin Space Grant Consortium's Undergraduate Research Fellowship.

**References**

Börnke, Frederik, and Thorsten Rocksch. 2018. "Thigmomorphogenesis – Control of Plant Growth by Mechanical Stimulation." Scientia Horticulturae 234: 344–53. doi: https://doi.org/10.1016/j.scienta.2018.02.059.

Choi, Won Gyu, Richard J. Barker, Su Hwa Kim, Sarah J. Swanson, and Simon Gilroy. 2019. "Variation in the Transcriptome of Different Ecotypes of Arabidopsis Thaliana Reveals Signatures of Oxidative Stress in Plant Responses to Spaceflight." American Journal of Botany 106 (1): 123–36. doi:10.1002/ajb2.1223.

Fitzgerald, Caleb, Cullen S Vens, Nathan Miller, Richard Barker, Matthew Westphall, Johnathan Lombardino, Jerry Miao, Sarah J Swanson, and Simon Gilroy. 2022. "Using the Automated Botanical Contact Device (ABCD) to Deliver Reproducible, Intermittent Touch Stimulation to Plants." Methods in Molecular Biology (Clifton, N.J.) 2368. United States: 81–94. doi:10.1007/978-1-0716-1677-2_6.

Hoson, Takayuki. 2014. "Plant Growth and Morphogenesis under Different Gravity Conditions: Relevance to Plant Life in Space." Life (Basel, Switzerland) 4 (2). Switzerland: 205–16. doi:10.3390/life4020205.

Monshausen, Gabriele B., and Elizabeth S. Haswell. 2013. "A Force of Nature: Molecular Mechanisms of Mechanoperception in Plants." Journal of Experimental Botany. doi:10.1093/jxb/ert204.

Schindelin J, Rueden CT, Hiner MC, Eliceiri KW. 2015. The ImageJ ecosystem: An open platform for biomedical image analysis. Molecular Reproduction and Development 82: 518–529.

Toyota, Masatsugu, and Simon Gilroy. 2013. "Gravitropism and Mechanical Signaling in Plants." American Journal of Botany 100 (1): 111–25. doi:10.3732/ajb.1200408.