Cantaurus, Vol. 25, 38-41, May 2017 © McPherson College Department of Natural Science

Thinopyrum intermedium Primary Root Growth Affected by Simulated Microgravity

David A. Nobo

ABSTRACT

Root gravitropism is the plant's ability to orient itself in response to the gravity vector. The gravitropic response of *Thinopyrum intermedium* was measured through the use of a 2-D clinostat. A 2-D clinostat is used to simulate a microgravity environment, allowing for the study of gravitropism and its effect on the primary root. Seeds were germinated for three days and were randomly plated in one of two groups: (1) 1g control or (2) simulated microgravity (µg), where they were subjected to 2rpm. Primary root growth between the two groups showed no difference in growth rate and, as expected, there was a significant change in root gravitropism. The results from this research suggest *Thinopyrum intermedium*'s primary root growth rate is not affected by a simulated microgravity environment.

Keywords: *Clinostat (two-dimensional), Gravitropism, Growth direction*, *Growth rate, Simulated microgravity, Thinopyrum intermedium.*

INTRODUCTION

Gravity is an abiotic stressor which plants depend on for directional root growth. One of the first to research and document these effects was Charles Darwin. In 1881, *The Power of Movement In Plants* was published. In this work, Darwin investigated various plant processes, including the gravitropic response of many species. In his experiments, seeds were grown and their radicles were placed upright at various angles. Measurements of radicle response were then taken over a course of several hours. The radicles changed their orientation from their initial upright position and oriented themselves with respect to the gravity vector (C. Darwin and F. Darwin 1881). Since then, there has been extensive research to understand the gravitropism of plants.

The starch-statolith hypothesis provides the current understanding of graviperception in plant roots. Statoliths are organelles in the cell responsible for the synthesis and storage of starch. Accumulation of statoliths on the wall of statocytes, the cells responsible for graviperception, causes a distinct growth of the radicle in the direction of the gravity vector ([UN]). Starchless mutants lacking phosphoglucomutase, an enzyme required for starch synthesis, showed an attenuated response to gravity due to lack of statoliths settling on the wall of the statocyte (Kiss et al. 1989). Consumption of the starch granules also attenuated graviperception in barley plants. Plants were kept in the dark for five days where the starch was consumed by respiration. This resulted in the barley plants completely losing their ability to sense gravity (Atwell et al. 1999). Further emphasis of the importance of the sedimentation of statoliths in the statocyte comes from removal of the root cap. *Arabidopsis* roots subjected to laser removal of defined cells in the root cap showed inhibited response to gravity, consistent with the starch-statolith hypothesis (Blancaflor et al. 1998).

Microgravity and simulated microgravity provide an ideal environment for studying gravitropic responses in plant roots. The International Space Station (ISS) provides a microgravity environment, making it an ideal venue for studying gravitropic response in plants (Herranz et al. 2013). Lentil seedling roots have been studied aboard the ISS where their ability to perceive gravity was tested and their root curvature was measured over a unit of time (Driss-Ecole et al. 2008). *Arabidopsis thaliana* has also been studied aboard the ISS, where it was successful in completing an entire seed-to-seed cycle (Link et al. 2003). Simulated microgravity experiments are carried out through the use of 1-D, 2-D, and 3-D clinostats (Herranz et al. 2013). These simulated microgravity machines provide similar results to microgravity experiments. Lentil seedlings were grown in two different environments: in microgravity and a slowly rotating clinostat. Researchers concluded that root length and orientation were similar to roots grown in microgravity and the clinostat, as well as statoliths were identically distributed in the statocytes (Lorenzi and Perbal 1990).

Microgravity causes an inhibited transport of auxin from cell to cell and subsequently causes attenuated cell growth in plant roots. *Arabidopsis* seeds germinated in the ISS and on a 3-D clinostat on earth showed an increase in cell proliferation in their roots, but an attenuation of cell growth (Medina and Herranz 2010). Plants grown in microgravity, real or simulated, exhibited an inhibition of auxin polar transport. Etiolated pea and maize seedlings grown

in microgravity aboard the STS-95 spacecraft showed similar results. Auxin polar transport was monitored in various segments of the plant and researchers concluded polar auxin transport, as well as plant growth and development, are mediated by earth's gravity (Ueda et al. 2000). Inhibition of auxin transport is seen directly in soy roots. Three soybean cultivars, each subjected to different temperatures: 18, 21, and 24ºC, were clinorotated on 1-D clinostat at a speed of 2rpm. When compared to a 1g control, the clinorotated group had smaller radicle lengths (De Micco et al. 2006).

The objectives of the research are to subject *Thinopyrum intermedium* to a simulated microgravity environment through the use of a 2-D clinostat, and measure the average growth rate and gravitropic response. Based on current literature findings, it is hypothesized plant roots subjected to simulated microgravity will be shorter in length compared to a 1g control, as well as having no preferential direction of growth when compared to a 90º rotated group.

MATERIALS AND METHODS

Petri plates and plant-agar preparation

Plant Agar (Duchefa Biochemie) was prepared according to the manufacturer's instructions, dispensed into 15mm by 100mm sterile plastic petri plates (Fisher Scientifics) in a laminar flow hood, and stored at 4ºC until used.

Seed preparation

Thinopyrum intermedium seeds were provided by Dr. Lee DeHaan at The Land Institute in Salina, KS. All seed preparation and handling was done in a laminar flow cabinet. Seeds were first surface sterilized in a 15% bleach solution for 12 minutes where they were then passed through a strainer. They were thoroughly washed five times with deionized water. Seeds were then placed in sterilized petri dishes – dimensions 15mm by 100 mm (Fisher Scientifics) – filled with deionized water where they were allowed to germinate for 72 hours in the dark to avoid phototropic stimuli.

Simulating microgravity: 2-D clinostat

The 2-D clinostat is provided by McPherson College. The specs of the equipment are as described in Table 1 ([UN]). Plates for the 1g control, 90° rotated group, and treatment group were prepared in similar fashion. Seeds were spaced evenly with as many viable seeds possible, leaving room for growth. Each seed is oriented in the same direction. 1g control plates are placed vertically, in alignment with the gravity vector, and sealed with Parafilm M® around the edges. The 90° rotated groups are made by simply turning the 1g control group 90°. Markings indicating the direction of the gravity vector, are made on the plates. The clinorotated groups are centered on the clinostat and held on the machine by tape. The groups subjected to the clinostat experienced a rotation rate of 2rpm. If rotational speeds are too high, samples will undergo centrifugal force, where the subjects will experience outward as down and will give skewed results when analyzed. Pictures of each group were taken every four-six hours for a period of two days and results for gravitropism and growth rate was recorded for analysis.

Analysis of results: ImageJ software

ImageJ is an open source software which can be downloaded free at http://rsb.info.nih.gov/ij/. Pictures of each group were saved to a computer and analyzed using the ImageJ software. Measurements of all curvature angles of the roots were taken using the angle measurement tool. Starting at the middle of the root tip, lines are created based on when the curvature begins. Once calculated, measurements were subtracted from 180º to get the true curvature angle. The same pictures will be used to analyze the growth rate of the roots. Initial lengths of the roots was measured using the segmented line tool and recorded in millimeters (mm) and the average growth rate was calculated (millimeters/hour). An independent samples t-test was used to determine if there's a statistical significance between the control and treatment groups for both variables.

RESULTS

Primary root growth

Surface sterilization of the seed populations using a 15% bleach solution was effective in reducing fungal growth. Primary root growth of *Thinopyrum intermedium* under 1g control and simulated microgravity (µg) conditions were compared. When both groups were compared, there was no significant difference in growth rates between the two growth environments.

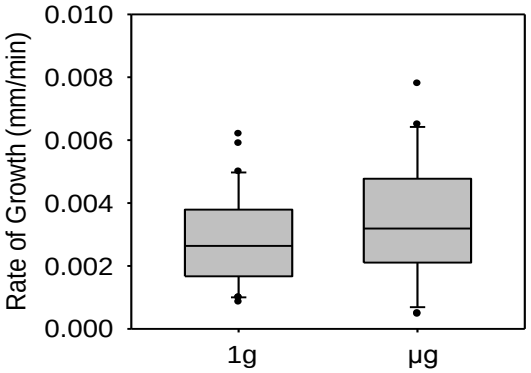

Figure 1. Effects of gravity (1g) and simulated microgravity (µg) on primary root growth rate. 1g n=32, µg n=22.

Root curvature

Seedlings rotated 90° and simulated µg group's root curvatures were measured and compared. Seedlings rotated 90° showed a distinct directional growth in response to a change in the gravity vector, averaging an angle of 61.8°. The simulated µg group showed no preferential growth towards the gravity vector, averaging a directional growth of 5.1°.

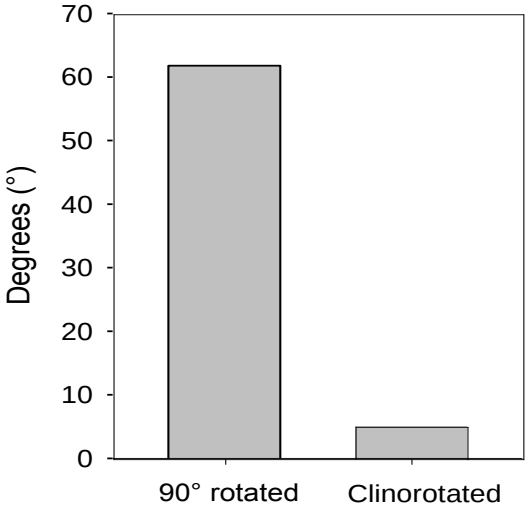

Figure 2. Average orientation of primary root directionality in each treatment measured in degrees. 90° rotated: 61.7° Clinorotated: 5.1°.

DISCUSSION

The 2-D clinostat represents an effective instrument for simulating a microgravity environment (Herranz et al. 2013). According to the results from this study, simulated microgravity did not attenuate primary root growth when compared to a 1g control (Figure 1). In the primary literature, different plant species respond differently to a simulated microgravity environment. For instance, soybean cultivars grown on a 1-D clinostat showed a significant attenuation of primary root growth, specifically those grown at 21°C. Other groups grown at 18°C and 24°C showed no significant difference in primary root growth (De Micco et al. 2006). While cress (*Lepidium sativum*), maize (*Zea mays*), rice (*Oryza sativa*), pea (*Pisum sativum*) were all subjected to a simulated microgravity environment by the use of a 3-D clinostat and exhibited no influence on the primary root growth rate, with the exception of the azuki bean (*Vigna angularis*), which showed a minimal increase in growth rate (Hoson et al). In this study, there proved to be no difference in growth rate in the two growth environments ($p > 0.05$).

There is a significant difference in the orientation of root growth direction. Plant roots in the µg group displayed a difference in gravitropism as compared to the 1g group which displayed normal gravitropic behavior (Figure 2). This can be attributed to the lack of sedimenting statoliths on the cell wall, which is essential for root orientation and curvature (Kiss et al. 1989).

There is a problem regarding the statistical power of our experimental design. With only n=32 in our 1g control and n=21 in our µg group, our data has a significantly low statistical power. A sample size of n=137 in each group would be required to get a desired power of 0.8. Future direction would include redplicating this research with the appropriate amount of samples to confirm or deny the results presented. In addition, the use of different dimensional clinostats has recorded variation in statolith distribution in columella cells located in the root cap (Kraft et al. 2000). Specifically, a random positioning machine, referred to as a 3-D clinostat displayed similar statolith distribution when compared to a microgravity environment, while a 2-D clinostat showed significant variation. Measuring the rate of growth using a 3-D clinostat would provide data from a more accurate microgravity environment.

ACKNOWLEDGEMENTS

I would like to thank Dr. Frye and Dr. Wilgers for their revisions of the paper and again, Dr. Frye for his patience, guidance, and indispensable input on the project. I would also like to thank the McPherson College department of Natural Science for their support and access to their resources and instruments/equipment. Finally, I would like to thank Dr. Lee DeHaan at the Land Institute in Salina, KS for providing the seeds for the experiment.

LITERATURE CITED

Atwell B, P. Kriedemann, and C. Turnbull. 1999. Plants in action. 1st ed. Melbourne (AU): Macmillian Education Australia.

Blancaflor E, J. Fasano, S. Gilroy. 1998. Mapping the functional roles of cap cells in the response of *Arabidopsis* primary roots to gravity. Plant Physiology 116(1):213-222.

Darwin C, and F. Darwin. 1881. The power of movement in plants. 1st ed. New York: D. Appleton and Company.

De Micco V, G. Aronne, and S. De Pascale. 2006. Effect of simulated microgravity on seedling development and vascular differentiation of soy. Acta Astronautica 58(3):139-148.

Driss-Ecole D, V. Legué, E. Carnero-Diaz, and G. Perbal. 2008. Gravisensitivity and autmorphogenesis of lenti seedling roots grown on board the International Space Station. Physiologia Plantarum 134(1):191-201.

Hoson T, S. Kamisaka, Y. Masuda, and M.

Yamashita. 1991. Changes in plant growth processes under microgravity conditions simulated by a three-dimensional clinostat. Journal of Plant Research 105(1): 53-70.

Herranz R, R. Anken, J. Boonstra J, M. Braun, P. Christianen, M. de Geest, J. Hauslade, R. Hilbig, RJ. Hill, and M. Lebert, et al. 2013. Ground-based facilities for simulation of microgravity: organism-specific recommendations for their use, and recommended terminology. Astrobiology 13(1):1-17.

Kiss JZ, R. Hertel, and F. Sack. 1989. Amyloplasts are necessary for full gravitropic sensitivity in roots of *Arabidopsis thaliana*. Planta 177(2):198-206.

Kraft TFB, JJWA. van loon, and JZ. Kiss. 2000. Plastid position in *Arabdidopsis* columella cells is similar in microgravity and on a random-position machine. Planta 211: 415-422.

Link BM, SJ. Durst, W. Zhou W, and B. Stankovic. 2003. Seed-to-seed growth of *Arabidopsis thaliana* on the International Space Station. Advances in Space Research 31(10):2237-2243.

Lorenzi G, and G. Perbal. 1990. Root growth and statocyte polarity in lentil seedling roots grown in microgravity or on a slowly rotating clinostat. Physiologia Plantarum 78(4):532-537.

Median FJ, and R. Herranz. 2010. Microgravity environment uncouples cell growth and cell proliferation in root meristematic cells. Plant Signaling & Behavior 5(2):176-179.

Ueda J, K. Miyamoto, T. Yuda, T. Hoshino, K. Sato, S. Fuiji, S. Kamigaichi, R. Izumi, N. Ishioka, and S. Aizawa, et al. 2000. STS-95 space experiment for plant growth and development, and auxin polar transport. Biological Sciences in Space 14(2):47-57.

[UN] United Nations Office for Outer Space Affairs. 2013. Programme on space applications teacher's guide to plant experiments in microgravity. New York: United Nations. www.unoosa.org/en/SAP/hsti/index.html (4 December, 2015).