Analysis of Development in Strains of the Plant *Arabidopsis thaliana* in Altered Gravity Conditions

Melika Osareh

Mentor: Dr. John Z. Kiss

University of North Carolina at Greensboro

Abstract:

To enhance current knowledge on growing plants in microgravity and reduced gravity conditions (e.g., Moon, Mars), this research project aimed to find strains of the model plant *Arabidopsis thaliana* that showed possible resistance to gravitational stress. Gravitational stress resistance was displayed in strains that demonstrated a similar growth response to the strains grown under normal conditions. In this project, 70 wild-type strains of *Arabidopsis thaliana* were tested for their gravitational stress-resistance by simulating microgravity with a 2-D clinostat, a device designed to randomize gravity. Surface-sterilized seeds were placed in Petri dishes with Murashige and Skoog nutrient agar and rotated on a clinostat or kept vertically (for controls) for seven days. The average shoot length, main root length, number of secondary roots, total root length, and number of root hairs per total root length of the clinorotated seedlings are compared to that of the vertically-grown controls using FIJI ImageJ, image-analysis tool. Under clinorotation, most strains showed significantly reduced shoot growth and varying growth in root and other parameters. Seven genotypes, however, were specifically of interest, including CIBC-5, which showed no statistically significant difference in growth across all parameters tested. We hypothesize that these genotypes will adapt better for growing in microgravity conditions and will be our prime candidates for further ground-based and spaceflight testing.

Introduction

Tropisms are adaptations in plants that allow them to respond to environmental factors such as differences in the availability or wavelength of light, water, minerals, and the gravity vector (Vandenbrink et al., 2014; Vandenbrink & Kiss, 2019). Plants have developed many adaptations to help them survive in stressful conditions here on Earth. Among these plant movements are tropisms, directed growth to external stimuli such as gravity (gravitropism) and light (phototropism) (Kaufman et al., 1987). However, there is a dearth of research on the molecular mechanisms of gravitropism. Thus, this research aims to aid in the understanding of this phenomenon. Other adaptations consist of resistance to droughts, increased soil salinity, and toxic metals (Nadeem et al., 2014).

Due to gravitropism on the Earth at 1-*g*, plant shoots normally grow against the gravity vector (upward; Fig. 1) and roots grow towards the gravity vector (downward) (Swatzell & Kiss, 2000; Vandenbrink & Kiss, 2019). In space at microgravity or at a reduced gravity, plants experience gravitational stress, which negatively affects plant growth (Kiss, 2014; Matía et al., 2010). During long-term space missions, astronauts depend on plants for food, oxygen, and psychological benefits (Wolverton & Kiss, 2009). Studying plant responses to the gravitational stress is particularly important for growing plants in space (Correll et al., 2013; Vandenbrink et al., 2014).

Natural plant strains vary in their resistance to stress factors. Thus, only stress-resistant plant varieties must be used to ensure the best growth and productivity in space or other space bodies such as the Moon or Mars (Kiss, 2014). Since the full genome of *Arabidopsis thaliana* plant has been sequenced, this plant serves as a suitable model for experimentation. If the genes that are responsible for adaptation to gravitational stress can be identified through our research, they could potentially be manipulated for plant varieties suitable for spaceflights. Since spaceflight experiments are very costly, initial studies often used so-called simulated microgravity via clinorotation (Kiss, 2015). The 2D-clinostat (Fig. 2) is a wheel like device rotating at about 1-2 rpm and randomize gravity vector (Kiss et al., 2019) . The goal of this research project is to use simulated microgravity through clinorotation to analyze 70 wild-type strains of *Arabidopsis thaliana* for their resistance to gravitational stress.

Methods:

This research project uses *Arabidopsis thaliana*, a common flowering plant from the mustard family, to study the effects that microgravity has on plant growth. Seeds for 70 wild-type strains were obtained from Arabidopsis Biological Resource Center (Columbus, OH). For experimentation, the external coat of seeds from each strain of *Arabidopsis thaliana* were surface sterilized using a 4% (w/v) bleach solution and then placed in square Petri Dishes (10 cm x 10 cm) under a laminar flow hood. The petri dishes were filled with a 1% (w/v) agar with Murashige and Skoog nutrients as medium. Six plates were prepared for each strain, with each plate containing 12 individual seeds distanced by 1.0 cm apart.

Of the six plates, three were labelled as "control" and three as "clinostat". After a 2 to 3-day period in the refrigerator (to stimulate uniform germination), the plates were placed vertically on a tray (control) and the experimental plates were placed on a clinostat, rotating at 1.25 rpm at 22^{o} C. After a 7day period, the plates were scanned using an EPSON high-resolution scanner (model #J252A), and then each strain was analyzed using the FIJI Image-J image analysis software (version #2). Each strain's gravitational stress resistance was determined using t-tests for each growth parameter (shoot length, main root length, total root length, number of secondary roots, and root hairs per total root length) comparing the means from the experimental group of that strain to its control group. Images of control and clinorotated seedlings are shown in Fig. 1. A baseline p-value of 0.05 was used to determine statistical significance, however, p-values of less than 0.01 were also be noted.

Shoot lengths were measured by measuring from the shoot apical meristem of the seedlings to the start of the root junction. Total root length was calculated by measuring from the root junction to the root tip and adding this measurement to the length of any secondary roots that were present. The number of secondary roots per total root length was calculated by first counting all the root hairs on a seedling, then dividing it by the total root length. For each genotype, the number of secondary roots per seedling between control and clinostat-treated were also noted and compared among other genotypes. The data for the number of secondary roots parameter was represented differently than other parameters due to the lack of continuity in the data, with some genotypes having no secondary roots in the control or treatment group (ie. Ct-1) which would pose a problem when taking percentages.

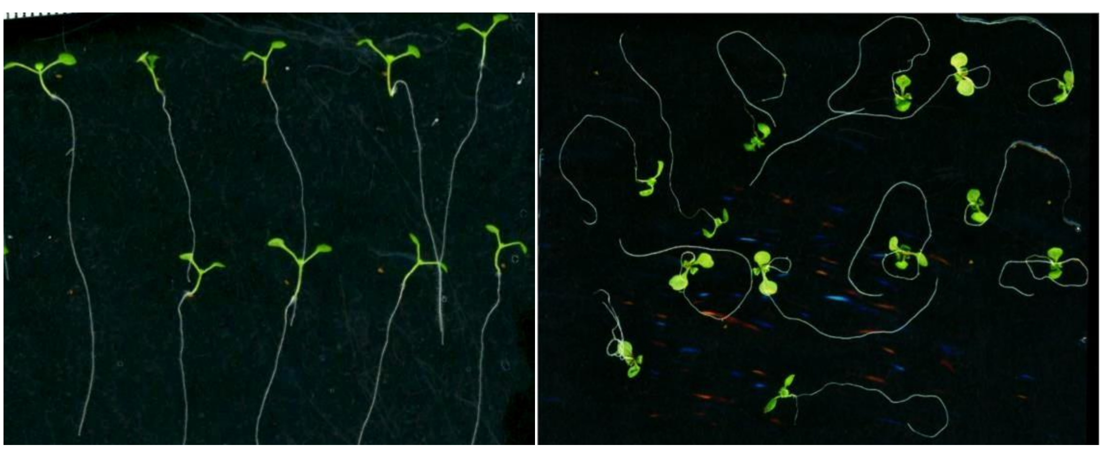

Figure 1: Control seedlings (left) and clinorotated seedlings (right).

Results:

In most cases, seedlings grown on a rotating clinostat had a significant decrease in shoot growth compared to the vertical controls. However, from the 70 genotypes analyzed, two genotypes, CIBC-5 and Col-0 showed no significant difference in shoot length under simulated gravitational stress from the vertically-grown controls. The other 68 genotypes had a significant decrease in shoot length compared to the controls (Fig. 2A). In their total root length, 32 genotypes showed no significant difference from controls. Eight genotypes showed a significant increase in growth and 30 genotypes showed a significant decrease in growth compared to the controls (Fig. 2B). In the number of root hairs per total root length, 20 genotypes showed no significant difference from controls, 3 genotypes showed a significant decrease, and 47 showed a significant increase in root hairs (Fig. 2C). Forty genotypes had no significant difference in the number of secondary roots, nine genotypes had significantly fewer secondary roots, and 21 had significantly more secondary roots as compared to their vertically grown controls (Fig. 3).

The genotype CIBC-5 was the only genotype found so far to show no difference in growth in <u>all of the parameters measured.</u> Apart from CIBC-5, five other genotypes, Sq-8, NFA-8, Omo2-3, Edi-0, Pna-10, showed no difference in growth among all growth parameters other than shoot length, and eleven genotypes showed no difference in growth in at least 3 parameters (Table 1).

Discussion:

As expected, the majority of seedlings grown under clinorotation had reduced growth parameters when compared to their vertically-grown controls. Most notably, shoot growth was negatively affected. Of the 70 *Arabidopsis thaliana* genotypes analyzed, 7 genotypes were of particular interest. CIBC-5, an ecotype from the United Kingdom region (Anastasio et al., 2011), showed no significant difference from controls among <u>all</u> growth parameters analyzed. Additionally, Col-0, an ecotype originally from Poland (Fernandez et al., 2018), was the only other genotype to not show a significant difference in shoot length. The Col-0 genotype also showed no significant difference in the number of secondary roots and root hairs per total root length. Furthermore, genotypes Sq-8, NFA-8, Edi-0, Omo-2-3, and Pna-10 all showed no significant difference in growth among all parameters analyzed except in their shoot lengths (Table 1), and may be ideal for further testing.

It is unknown whether clinorotation is a true prediction for growth response at microgravity conditions. In order to fully understand plant tropisms, experiments in true microgravity or altered gravity conditions must be performed. Nonetheless, genotypes such as Col-0 have also been identified by prior research as having possible gravitational resistance qualities, especially in root growth, based on spaceflight experiments (Paul et al., 2017) .

Prime candidates for testing on spaceflight experiments include genotypes that display possible gravitational stress resistance in phenotypic traits. This trait may be exemplified by genotypes that show similar growth under simulated gravitational stress by a 2D-clinostat. Therefore, the seven genotypes listed above, and particularly CIBC-5, will be our main candidates for further testing here on Earth and possibly in future spaceflight experiments. Selected candidates will have genetic studies performed and compared in order to identify possible genes responsible for gravitational stress resistance. Later, these genes can be checked or manipulated to produce plant varieties suitable for gravitational stress resistance.

While studies have shown that genotypes such as Col-0 can be made to adapt to spaceflight conditions through alteration of a single gene and several other methods to maintain root growth (Paul et al., 2017), most studies have not assessed shoot length and other parameters discussed in this research which can be important factors in a plant's response to gravity. Spaceflight studies which have focused on phototropism, however, found no significant difference in growth between microgravity and 1-g treatments in *phytochrome A*, *phytochrome B*, and wildtype (Landsberg; Ler) genotypes (Vandenbrink & Kiss, 2016). Additionally, most studies have focused on using Landsberg (Ler), Wassilewskija (WS), or phytochrome mutant genotypes of *Arabidopsis thaliana* (Vandenbrink & Kiss, 2016). Therefore, we aimed to use genotypes from a broad range of locations to expand on the current knowledge on *A. thaliana*'s growth responses. Understanding the growth responses of more genotypes under gravitational stress provides for better assessment of said results for selecting candidates to alter for spaceflight experiments and genetic modification.

Spaceflight research comes with many challenges such as limitations in resources, the lack of a proper control group, limitations in sample size, safety concerns, and the effective processing and analysis of samples after spaceflight among many others (Kiss, 2015). Due to the many constraints of such experiments, development of effective ground-based models are an important part of furthering space biology research and ensuring the enhancement of spaceflight experiments when conducted. Learning from past experiments (e.g., Kiss, 2014) for lighting and temperature and others such as determining a clinostat rotational velocity so as to prevent difficulty in root nutrient uptake shown to be a problem at higher velocities (Polinski et al., 2017). The procedures used in this experiment were optimized to best determine the several growth parameters of *A. thaliana* under gravitational stress using a 2D-clinostat. Spaceflight experiment results can be compared to results from this experiment to determine the effectiveness of a 2D-clinostat in randomizing the gravity vector for plants.

Conclusions:

Based on 2D-clinorotation treatment, CIBC-5, an *A. thaliana* genotype, is expected to be more tolerant at gravitational acceleration stress conditions among all growth parameters. Six other candidates mentioned also show similar results. Further tests using other gravity-randomizing models such as the random-positioning machine (RPM) could be used to verify results from this experiment (Kiss et al., 2019). Additionally, more testing will be needed to find other genotypes that may show vigorous growth under gravitational stress. In the long term, experiments in true microgravity conditions need to be performed to prove or disprove our predictions based on clinorotation treatment.

Acknowledgements:

Melika Osareh carried out portions of this research along with support from lab mates Alexandra Settle, Alena Jones, Noah McMurry, Kelsey Taylor, and Ibeabuchi Iloghalu with supervision from postdoctoral fellow Dr. Tatsiana Shymanovich in Dr. John Z. Kiss' lab. Melika's contributions were fulfilled in part through requirements of Biology 499 Undergraduate Research course and the Biology 493 Honors Undergraduate Research course at the University of North Carolina at Greensboro, as well as the NASA North Carolina Space Grant Undergraduate Research Award in summer 2019. Additional funding for this research was through Undergraduate Research Award from the Department of Biology in 2019 at the UNC-Greensboro to Melika as well as NASA grant funding to Department of Biology for Dr. John Z. Kiss.

Figures and Tables:

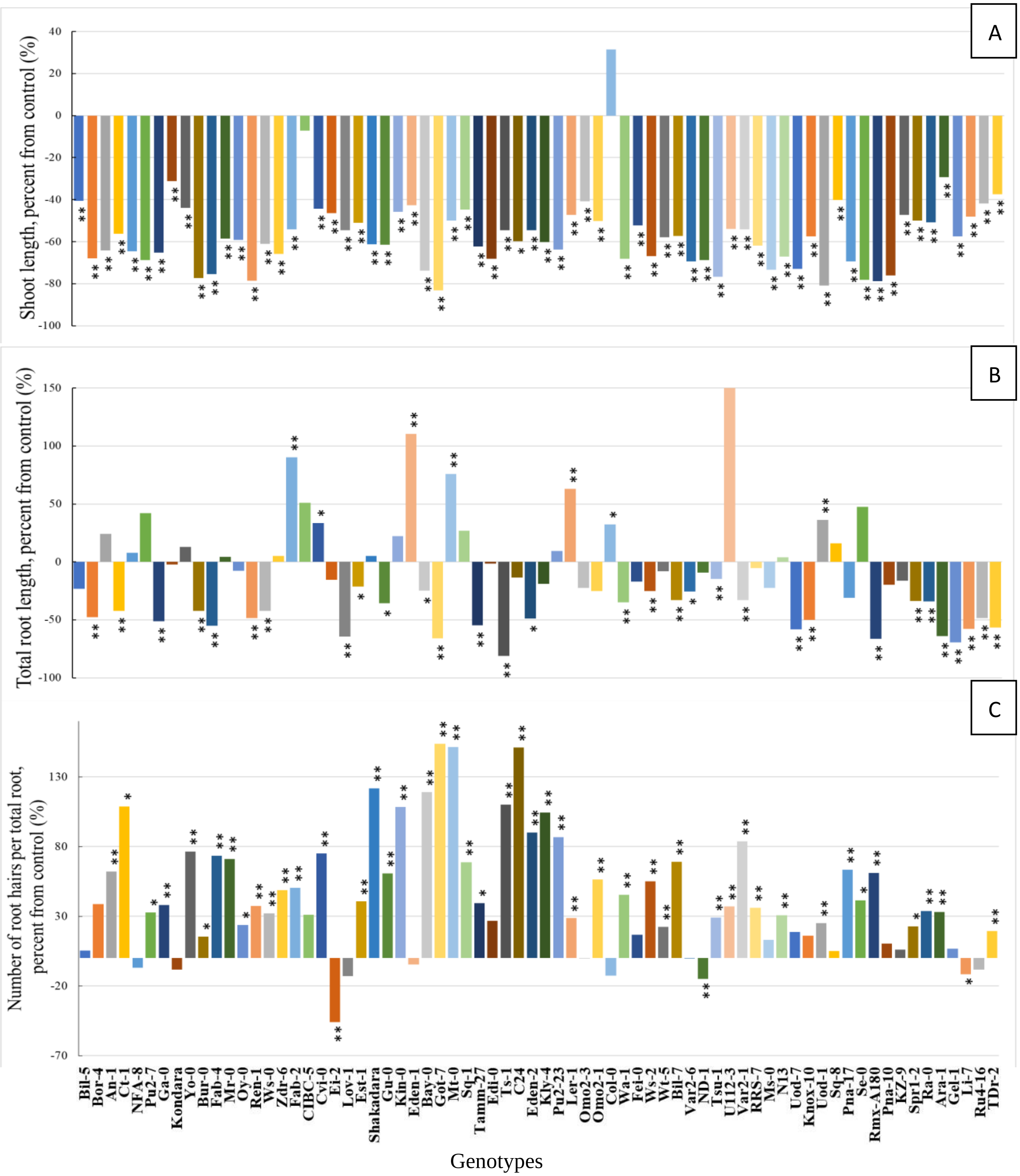

***Figure 2**: Comparison of seedling growth parameters for shoot length (A), total root length (B), and number of root hairs per total root length (C) for clinorotated versus vertically grown control seedlings for 70 Arabidopsis thaliana wildtype genotypes. Each parameter is shown as (mean clinorotated / mean control * 100) - 100. Asterisks indicate t-test p-values of < 0.05 (*), or < 0.01 (**).*

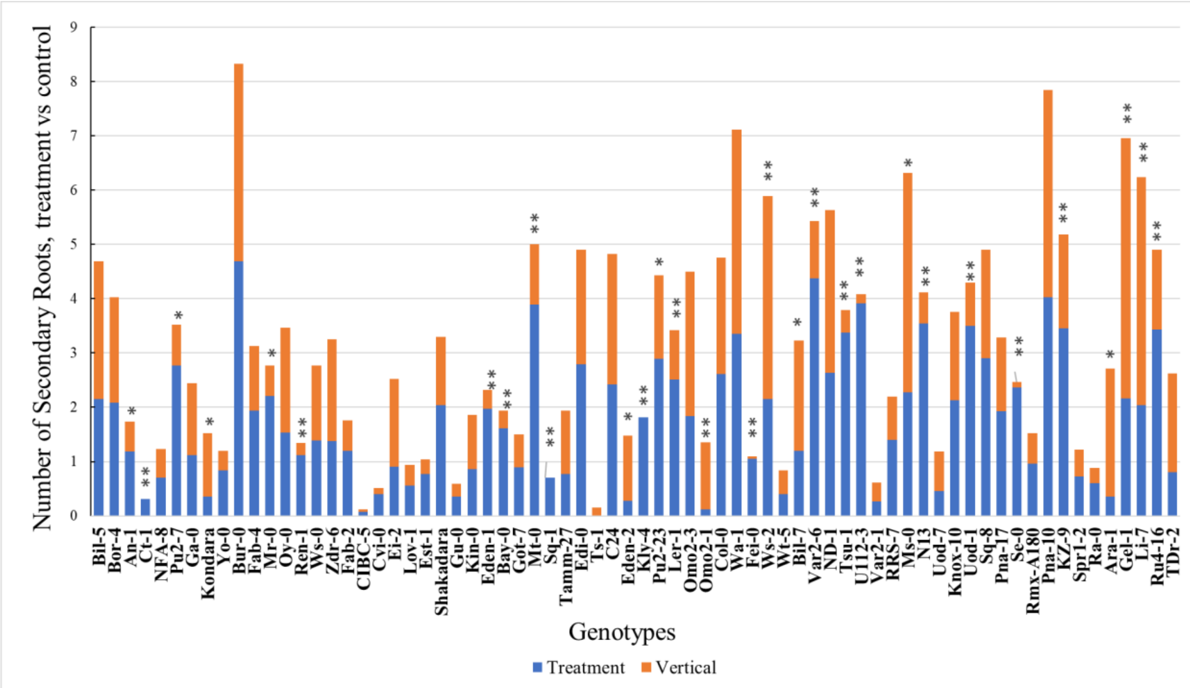

Figure 3: *Comparison of average number of secondary hairs for clinorotated treatment group versus vertically-grown control group in 70 Arabidopsis thaliana wildtype genotypes. Asterisks indicate t-test p-values of < 0.05 (*), or < 0.01 (**).*

***Table 1**: Analysis of development of genotypes (strains) in altered gravity conditions compared to the control. Rows show genotypes in decreasing order of number of parameters (ranked A-F) with nonsignificant p-values (<0.05; NS) among 5 different parameters (shoot length, main root length, number of secondary roots, total root length, and number of root hairs per total root length) in vertical versus clinorotated seedlings. While there are more genotypes in the D-F categories, these genotypes shaded in dark grey are included for reference. *Genotype Col-0, while in tier C, is notable due to being one of the only two genotypes with nonsignificant shoot growth under clinorotation compared to controls.*

Genotype	Shoot Length	Main Root Length	Number of S. Roots	Total Root Length	NRH/TRL	Tier
CIBC-5	NS	NS	NS	NS	NS	A
Col-0	NS	S	NS	S	NS	C*
Sq-8	S	NS	NS	NS	NS	B
NFA-8	S	NS	NS	NS	NS	B
Omo2-3	S	NS	NS	NS	NS	B
Edi-0	S	NS	NS	NS	NS	B
Pna-10	S	NS	NS	NS	NS	B
Kondara	S	NS	S	NS	NS	C
Pna-17	S	NS	NS	NS	S	C
Yo-0	S	NS	NS	NS	S	C
Oy-0	S	NS	NS	NS	S	C
Zdr-6	S	NS	NS	NS	S	C
SHAKADARA	S	NS	NS	NS	S	C
GU-0	S	NS	NS	NS	S	C
Kin-0	S	NS	NS	NS	S	C
C24	S	NS	NS	NS	S	C
ND-1	S	NS	NS	NS	S	C
RRS-7	S	NS	NS	NS	S	C
Fei-0	S	S	S	NS	NS	D
Omo2-1	S	NS	S	NS	S	D
Ws-0	S	S	NS	S	S	E
Tamm-27	S	S	NS	S	S	E
Ler-1	S	S	S	S	S	F

Literature Cited

Anastasio, A. E., Platt, A., Horton, M., Grotewold, E., Scholl, R., Borevitz, J. O., Nordborg, M., & Bergelson, J. (2011). Source verification of mis-identified Arabidopsis thaliana accessions: Mis-identified A. thaliana accessions. *The Plant Journal*, *67*(3), 554–566. https://doi.org/10.1111/j.1365-313X.2011.04606.x

Correll, M. J., Pyle, T. P., Millar, K. D. L., Sun, Y., Yao, J., Edelmann, R. E., & Kiss, J. Z. (2013). Transcriptome analyses of Arabidopsis thaliana seedlings grown in space: Implications for gravity-responsive genes. *Planta*, *238*(3), 519–533. https://doi.org/10.1007/s00425-013-1909-x

Fernandez, V., Barnaby, J. Y., Tomecek, M., Codling, E. E., & Ziska, L. H. (2018). Elevated CO_2 may reduce arsenic accumulation in diverse ecotypes of *Arabidopsis thaliana*. *Journal of Plant Nutrition*, *41*(5), 645–653. https://doi.org/10.1080/01904167.2017.1415352

Kaufman, P. B., Song, I., & Pharis, R. P. (1987). Gravity Perception and Response Mechanism in Graviresponding Cereal Grass Shoots. In S. S. Purohit (Ed.), *Hormonal Regulation of Plant Growth and Development* (pp. 189–200). Springer Netherlands. https://doi.org/10.1007/978-94-015-3950-0_10

Kiss, J. Z. (2014). Plant biology in reduced gravity on the Moon and Mars. *Plant Biology*, *16*, 12–17. https://doi.org/10.1111/plb.12031

Kiss, J. Z. (2015). Conducting Plant Experiments in Space. In E. B. Blancaflor (Ed.), *Plant Gravitropism* (Vol. 1309, pp. 255–283). Springer New York. https://doi.org/10.1007/978-1-4939-2697-8_19

Kiss, J. Z., Wolverton, C., Wyatt, S. E., Hasenstein, K. H., & van Loon, J. J. W. A. (2019). Comparison of Microgravity Analogs to Spaceflight in Studies of Plant Growth and Development. *Frontiers in Plant Science*, *10*, 1577. https://doi.org/10.3389/fpls.2019.01577

Matía, I., González-Camacho, F., Herranz, R., Kiss, J. Z., Gasset, G., van Loon, J. J. W. A., Marco, R., & Javier Medina, F. (2010). Plant cell proliferation and growth are altered by microgravity conditions in spaceflight. *Journal of Plant Physiology*, *167*(3), 184–193. https://doi.org/10.1016/j.jplph.2009.08.012

Nadeem, S. M., Ahmad, M., Zahir, Z. A., Javaid, A., & Ashraf, M. (2014). The role of mycorrhizae and plant growth promoting rhizobacteria (PGPR) in improving crop productivity under stressful environments. *Biotechnology Advances*, *32*(2), 429–448. https://doi.org/10.1016/j.biotechadv.2013.12.005

Paul, A.-L., Sng, N. J., Zupanska, A. K., Krishnamurthy, A., Schultz, E. R., & Ferl, R. J. (2017). Genetic dissection of the Arabidopsis spaceflight transcriptome: Are some responses dispensable for the physiological adaptation of plants to spaceflight? *PLOS ONE*, *12*(6), e0180186. https://doi.org/10.1371/journal.pone.0180186

Polinski, E., Schueler, O., Krause, L., Wimmer, M. A., Hemmersbach, R., & Goldbach, H. E. (2017). 2-D clinorotation alters the uptake of some nutrients in Arabidopsis thaliana. *Journal of Plant Physiology*, *212*, 54–57. https://doi.org/10.1016/j.jplph.2017.01.011

Swatzell, L. J., & Kiss, J. Z. (2000). Journey towards the centre of the earth: Plant gravitropism. *Biologist (London, England)*, *47*(5), 229–233.

Vandenbrink, J. P., & Kiss, J. Z. (2016). Space, the final frontier: A critical review of recent experiments performed in microgravity. *Plant Science*, *243*, 115–119. https://doi.org/10.1016/j.plantsci.2015.11.004

Vandenbrink, J. P., & Kiss, J. Z. (2019). Plant responses to gravity. *Seminars in Cell & Developmental Biology*, *92*, 122–125. https://doi.org/10.1016/j.semcdb.2019.03.011

Vandenbrink, J. P., Kiss, J. Z., Herranz, R., & Medina, F. J. (2014). Light and gravity signals synergize in modulating plant development. *Frontiers in Plant Science*, *5*. https://doi.org/10.3389/fpls.2014.00563

Wolverton, C., & Kiss, J. (2009). An update on plant space biology. *Gravit. Space Biol. Bull.*, *22*.