The Effects of Nitrogen Fixation in *Medicago sativa* Microgravity

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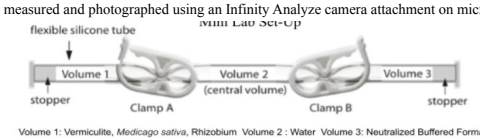
Abstract

Nitrogen Fixation is an important biological relationship that allows plants to utilize atmospheric nitrogen. Plants, such as *Medicago sativa*, allow rhizobium bacteria to infiltrate their root hairs; wherein these bacteria a enzyme to transform N2 to N3 to be used by the host plants (1). This experiment explored the effects that variation in microgravity has on this relationship. Conducting it using the *Medicago sativa* plant and three standard gravity replicates, and one microgravity replicate

Experimental Design

This experiment was conducted with a silicone tube apparatus and two clamps . The apparatus was cleaned using isopropyl alcohol in a sterile fume hood to avoid bacterial contamination. The clamps created three distinct sections that allowed for the flight experiment to be initiated once in microgravity. The first stopper was applied and the contents of the first section (Volume 1) where loaded into the cleaned apparatus. The first section contained autoclaved vermiculite (Sta-Green Organics), a synthetic soil, 4 *Medicago sativa* seeds and 0.2 ml *Rhizobium legumin Sarum* bacteria. The seeds, from (Visjon Biologicals), where dipped into isopropyl alcohol and allowed to dry in a sterile fume hood. Clamp A was then placed, and 2 ml distilled water (Sigma Aldrich), that had been run through a .01µm filter, was placed in second section using a syringe. Clamp B was then applied to the tube and 2 ml of NBF(Sigma Aldrich), was added into the third section and the final stopper was placed. The flight tube was then shipped to the launch site to be sent to the International Space station, and the ground experiments remained in the lab. The experiment was initiated by releasing clamp A and gently shaking the tube, allowing water into the first section. This began the process of germination. After 2 months, Clamp B was released, and the container was shaken to stop growth and preserve the plant structures.Once the Flight experiment returned to earth, all sprouts and root fragments were removed from the tube and placed into more NBF solution. Each replicates sprouts, nodules and root structures were measured and photographed using an Infinity Analyze camera attachment on microscope.



Volume 1: Vermiculite, *Medicago sativa*, Rhizobium Volume 2 : Water Volume 3: Neutralized Buffered Formalin

Conclusions

In conclusion, in this study we were able to identify differences in *Medicago sativa's* development and nodulation in microgravity and standard gravity. We found a statistically significant effect of sprout length and width on replicate type. A study of a larger scale, perhaps one that can incorporate light, would be beneficial for helping to create more sustainable space flight. Understanding the effects of microgravity on processes like nitrogen fixation is key to developing systems for to foreign soil development and sustainable subsistence agriculture in space.

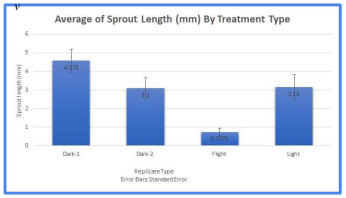
Results

To conduct a statistical analysis, I used the program R-studio. I used a two-way anova analysis. I found a significant effect of sprout length and width on replicant type (F = 1.0195, P = 0.4183)

As demonstrated in graph (1), the flight experiment incurred the least amount of development and averaged the shortest sprout length. Additionally, the flight experiment did see a lower percentage of nodulation then the light and one of the dark replicates. However it did match the percentage of the other dark replicate.

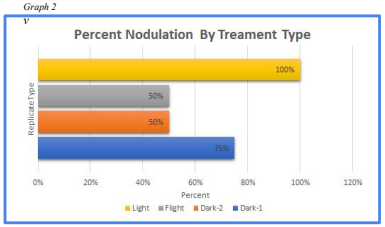
Sprout Development

Graph 1



Nodulation

Graph 2



Leaf Development

Light (Ground)

Figure 3

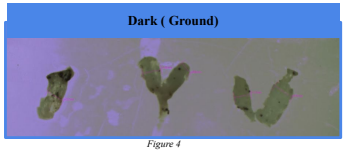


Figure 4

Flight (Dark)

Figure 5

Differences in Nodulation

Light

Figure 1

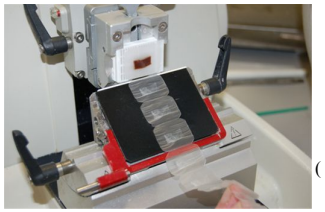
Flight

Figure 2

In Figure (1) and (2) you can visually see the differences in nodule and root formation under the duress of microgravity. The Flight sample figure (2) is much shorter and bunched together. These differences are also highlighted in figures (3, 4 and 5) where in the leaf development is distinctly different in the ground and flight trials. With the flight trials being smaller and more condensed.

Future Research

I plan on continuing this research by further examining formation of nodules in the flight and ground replicates. I plan on analyzing the root systems of the samples will be examined using components of cytology. Samples will be treated with a series of alcohols and paraffin waxes and a microtome will be used to create cross sections. Additionally, these sections will be stained with a series of dyes known to work well for rhizobium bacteria such as Ammonium oxalate crystal violet and Methylene blue. This will be used on nodules to determine the differences in infection in the flight and ground replicates.



(2)

References and Acknowledgements:

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References:

(1) Flynn, Robert, and John Idowu. "Nitrogen Fixation by Legumes." NMSU. College of Agricultural, Consumer and Environmental Sciences, New Mexico State University, June 2015. https://aces.nmsu.edu/pubs/_a/A129/.

(2) Donald B. McMillan, Richard J. Harris. (n.d.). *Microtome*. Microtome - an overview | ScienceDirect Topics. https://www.sciencedirect.com/topics/neuroscience/microtome.

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