International Journal of Biomedical and Health Sciences
Vol. 17, No.1, March 31, 2021
Printed in Nigeria

0794-4748/2021 $5.00 + 0.00

http://journals.niseb.org.ng

IJBHS 2020077/17104

# Space as a classic environment for microscopic and anatomical studies

**Funmilola A. Oluwafemi*[1,2], Elizabeth Aregbe[1], Omede Ameh[1], Omodele Ibraheem[2], Funmitan Oyetunde[1], Empress O. Okhuelegbe[1], Ropo A. Olubiyi[1], Oluwafemi A. Adeleke[3], Olayemi A. Adeniyi[4], Lekan Oluwafemi[3], Lekan Oluwafemi[3], Olufemi A. Agboola[1]**

[1]National Space Research and Development Agency (NASRDA), Abuja, Nigeria.
[2]Department of Biochemistry, Federal University Oye-Ekiti (FUOYE), Ekiti-State, Nigeria.
[3]NASRDA Cooperative Information Network (COPINE), Advanced Space Technology Applications Laboratory (South West), Obafemi Awolowo University, Ile-Ife, Osun state, Nigeria.
[4]IBERS, Aberystwyth University, Wales, United Kingdom.

*Contact: oluwafemifunmilola@gmail.com +2348065035799

(Received November 22, 2020; Accepted January 4, 2021)

ABSTRACT: One of the major characteristics of the space environment is microgravity. Space environment is now classified as a classic environment because it has constantly proved to be an outstanding environment giving high quality products. Microbiologists study the behavior of microorganisms in the space environment as microorganisms can form biofilm which are mainly antibiotics resistant for playing essential roles in human health for novel therapeutics and vaccines. Microscopic examination provides preliminary and tentative identification of cells (plant or animal). It reveals cell size, shape and structure. It is known from plant biology that plants have parenchyma cells that: make up plant structures including stems, roots, and leaves; they may be specialized to function in photosynthesis, storage, or transport; and they provide route of exchange for materials within and between the xylem and the phloem. Plants roots are structures specialized for anchorage, storage, absorption and conduction. Plants roots-anatomy are very important for gravi-responses and in plant physiology generally. In this study, peanut seeds were grown under normal earth gravity and under simulated microgravity environment – using clinostat, a microgravity simulations equipment at the Microgravity Simulations Laboratory, National Space Research and Development Agency (NASRDA), Abuja, Nigeria. Using the plant roots, the result showed that there was longer root lengths of the peanut seeds under the simulated microgravity environment than the seeds under normal earth gravity which served as the control ($P<0.05$). There were also the microscopic examinations of the roots of both samples. This paper discusses the specific differences in the roots anatomy including the root cells of these samples as viewed under the light microscope; and the specific deductions that could be made.

**Keywords**: Space, Microgravity, Microbiology, Plant, Clinostat.

## Introduction

Space environment is classified as a classic environment because it has constantly proved to be an outstanding environment giving high quality products. One of the major characteristics of the space environment is microgravity. Microgravity environment has a great impact on plant growth and development and it eventually affects plant yield (Oluwafemi *et al*., 2018). Microscopic examination provides preliminary and tentative identification of cells (plant or animal). Microorganisms are living microbes that cannot be seen without an aid of a microscope. The structure of cells are studied using microscope.

Plants roots anatomy are very important for gravi-responses and in plant physiology generally. In plants, parenchyma cells make up plant structures including stems, roots, and leaves; they may be specialized to function in photosynthesis, storage, or transport. This aim of this study was to determine the effects of simulated microgravity on the root anatomy of peanut. The specific objectives were to determine the differences: in the root lengths of peanut seeds grown under the normal earth gravity and in simulated microgravity environment; as viewed under the microscope, the cells of peanut root cells of seeds grown under the normal earth gravity and in simulated microgravity environment. The structural differences in the shape of the peanut root cells under the simulated microgravity environment and under normal earth gravity can only be determined using the microscope. Some specific benefits of this study was also discussed.

The aims and objectives in this study were achieved by an experimental example that was carried out at the Microgravity Simulations Laboratory of the Engineering and Space Systems Department (ESS) of National Space Research and Development Agency (NASRDA), Abuja, Nigeria.

Peanut is also known as groundnut, goober or monkey nut. It is taxonomically classified as *Arachis hypogaea* and technically considered as pea; it belongs to the family *fabaceae* of legume (Baughman *et al*., 2015). The properties that makes peanut suitable for this experiment are that it is small, easy to handle and fast-growing with germination time not longer than 3 days.

A Clinostat is an equipment that eliminates the effect of gravity; or it can be said to mimic microgravity. The Clinostat (Fig 1) that was used for this research is a One-Axis Clinostat (Desk-top type). Being a one axis clinostat means it is a two-dimensional (2-D) clinostat with a single rotational axis, which runs perpendicular to the direction of the gravity vector. It operates with respect to speed and direction of the rotation. A rotation on a clinostat is called “clinorotation” (United Nations 2013).

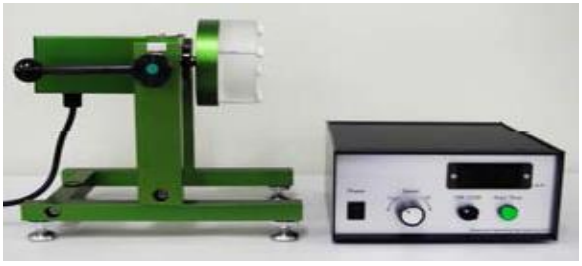

Fig 1: The Clinostat

## Materials and Methods

The substrate of the seeds called plant agar-agar was prepared into 2 petri dishes following the standard preparation method (1.5g into 100ml of tap water and heat till boiling) (United Nations, 2013), then the seeds were planted in the substrate and it was cultivated inside a wet chamber (Fig 2) in vertical positions. This is because plants grow vertically under normal Earth gravity. After 3 days, germination of the seeds with short roots was observed. The 2 petri dishes were then taken and labeled "1g-control" and "Clinorotated". The 1g-control sample was remained in the vertical position and the Clinorotated sample was then placed at the centre of the clinostat using double-sided tape. The Clinorotated sample was clinorotated for 3 hrs and pictures of the two samples were taken every 30 minutes during this observation. These pictures served to generate data.

Fig 2: The samples in the wet chamber

These observations were done under humidity between 60% to 100%, temperature of 23ºC (room temperature) and light of 50lux. In addition to these, the clinorotated sample had the following conditions, rotation speed of 85rpm, rotational-axis angle was horizontal and the direction of rotation was clockwise. At the end of observation, microscopic examinations were done on the roots of both samples.

### Statistical analysis of data

All the data were analyzed using Microsoft Excel and GraphPad prism 6. Values represent mean data ± the standard error of mean (Mean ± SEM). Student's t-test was used to determine the level of significance between two groups, whereas, two-way ANOVA was used for comparison between the two independent variables, 1g sample and clinorotated sample. $P<0.05$ was used as criteria for statistical significance.

## Results and Discussion

### Roots Length and Growth Rates

It was observed that the Clinorotated sample's roots (Fig 3) grew longer than the 1g-control sample (Fig 4). It was also observed that the roots of the 1g-control sample grew vertically downwards (Fig 4) while the roots of the Clinorotated sample were haphazard (Fig 3).

Further analyzes was done on the pictures of the samples using imageJ software. The root lengths of each of the picture taken were measured on the imageJ software. Then, the average of the lengths of the roots per picture was done. Finally, the growth rates of the two samples were determined.

Fig 3: Clinorotated Sample with Longer Roots

Fig 4: 1g-control Sample with Shorter Roots

**Table 2: Grand Averages of the Roots length of the 1g-control and Clinorotated Samples of Peanut**

| **Time (Hrs)** | **1g-Control** | **Clinorotated** |
|---|---|---|
| 0 | 8.08±1.42 | 9.89±1.67 |
| 0.5 | 8.62±1.64 | 9.52±1.57 |
| 1 | 8.57±1.41 | 9.62±1.55 |
| 1.5 | 8.22±1.39 | 10.75±1.47 |
| 2 | 8.56±1.28 | 11.28±1.26 |
| **Average±SEM** | **8.411±0.11** | **10.21±0.34** |

Since the plant was examined for 2 hours, therefore, the growth rate of the 1g-control sample is: 8.411228371 / 2 = 4.20mm/hr.

The growth rate of the Clinorotated sample is: 10.212925 / 2 = 5.11mm/hr. Comparing 1g to the clinorotated $P<0.05$

**Structural Analyzes**

Figs 5 and 6 are the view of the roots of the two samples under X40 objectives of the microscope.

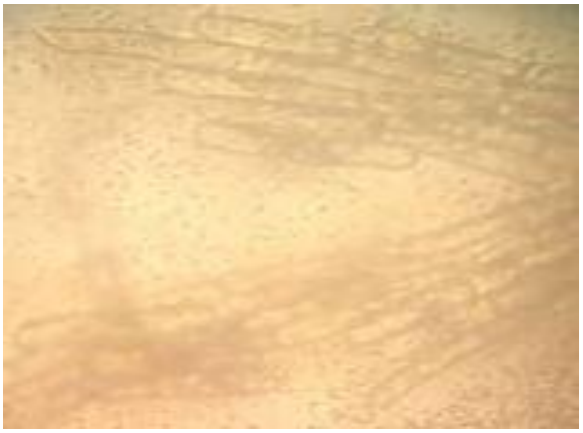

Fig 5: 1g-control Sample Microscopic Examinations

Fig 6: Clinorotated Sample Microscopic Examinations

**Conclusion**

It can be deduced that there are physiological basis that caused the root lengths of the Clinorotated sample to be longer than those of the 1g-control sample (Oluwafemi *et al*., 2020). These physiological bases could be that: the root cells were proliferating at a higher rate; there was an accelerated cell cycle (Howard, 2010). Auxin is a plant hormone that affect cell division and elongation in stems and roots. Auxin also regulate cell expansion in plants responses to light and gravity. Auxin is usually transported to its site of action. Therefore, it could be said that auxins were transported in larger quantity to the site of action in the Clinorotated sample than in the 1g-control sample.

It can be deduced from the microscopic examinations that there are changes in vascular structure as a result of the orientation of microfibrils and their assembly in developing vessels perturbed by microgravity**.**

**Acknowledgements**

United Nations Office for Outer Space Affairs (UNOOSA) in Vienna, Austria for contributing Clinostat to National Space Research and Development Agency (NASRDA), Obasanjo Space Centre, Abuja, Nigeria. The authors are also thankful for the research space created by the Department of Engineering and Space Systems Department, NASRDA.

## References

Baughman, TA., Woodward JE., Baring, MR., and Simpson, CE. 2015. Comparison of three high-oleic Peanut cultivars under varying field conditions in the southwestern United States. Peanut Science; Vol.42, No 1, pp.11-17. https://doi.org/10.3146/0095-3679-42.1.11.

Howard, GL. 2010. The influence of microgravity on plants. NASA Surface Systems Office, Space Life Sciences Laboratory, Mail Code NE-S-1, Kennedy Space Center, FL 32899. NASA ISS Research Academy and PreApplication Meeting, South Shore Harbour Resort & Conference Center, League City, Texas.

Oluwafemi, FA., De La Torre, A., Afolayan, EM., Olalekan-Ajayi, BM., Dhital, B., Mora-Almanza, JG., Potrivitu, G., Creech, J., and Rivolta A. 2018. Space food and nutrition in a long-term manned mission. Springer: Advances in Astronautics Science and Technology. https://doi.org/10.1007/s42423-018-0016-2.

Oluwafemi, FA., Ibraheem, O., Fatoki T.H. 2020. Clinostat microgravity impact on root morphology of selected nutritional and economic crops. Plant Cell Biotechnology and Molecular Biology 21(43&44):92-104. ISSN: 0972-2025.

United Nations. 2013. Teacher's guide to plant experiments in microgravity. Human Space Technology Initiative. United Nations Programme on Space Applications, New York. Publishing production: English, Publishing and Library Section, United Nations Office, ST/SPACE/63.