World Academy of Science, Engineering and Technology
International Journal of Bioengineering and Life Sciences
Vol:11, No:5, 2017

Growth and Anatomical Responses of *Lycopersicon esculentum* (Tomatoes) under Microgravity and Normal Gravity Conditions

Gbenga F. Akomolafe, Joseph Omojola, Ezekiel S. Joshua, Seyi C. Adediwura, Elijah T. Adesuji, Michael O. Odey, Oyinade A. Dedeke, Ayo H. Labulo

***Abstract*—**Microgravity is known to be a major abiotic stress in space which affects plants depending on the duration of exposure. In this work, tomatoes seeds were exposed to long hours of simulated microgravity condition using a one-axis clinostat. The seeds were sown on a 1.5% combination of plant nutrient and agar-agar solidified medium in three Petri dishes. One of the Petri dishes was mounted on the clinostat and allowed to rotate at the speed of 20 rpm for 72 hours, while the others were subjected to the normal gravity vector. The anatomical sections of both clinorotated and normal gravity plants were made after 72 hours and observed using a Phase-contrast digital microscope. The percentage germination, as well as the growth rate of the normal gravity seeds, was higher than the clinorotated ones. The germinated clinorotated roots followed different directions unlike the normal gravity ones which grew towards the direction of gravity vector. The clinostat was able to switch off gravistimulation. Distinct cellular arrangement was observed for tomatoes under normal gravity condition, unlike those of clinorotated ones. The root epidermis and cortex of normal gravity are thicker than the clinorotated ones. This implied that under long-term microgravity influence, plants do alter their anatomical features as a way of adapting to the stress condition.

***Keywords*—**Anatomy, Clinostat, Germination, Microgravity, *Lycopersicon esculentum*.

I. Introduction

MICROGRAVITY is a characteristic of a space environment, a condition of altered gravity which poses an abiotic stress on organism metabolism, growth and development. Various platforms have been used to simulate microgravity conditions, such as the dropping tower at the centre for microgravity research in Bremen, Germany, the suborbital flight usually funded by national space agencies across the world, and the clinostat. The International Space Station (ISS) has been an experimental platform to conduct research in true microgravity conditions. Several authors have simulated plants response to the condition of altered gravity [1]-[4].

Gravity is a fundamental force that affects everything on Earth according to the relation given by Sir Isaac Newton in (1):

$$F = G\,{M_1 M_2}/{r^2} \tag{1}$$

The clinostat simulate a fraction of the Earth's gravity when a biological system is subjected to horizontal rotation on it according to the relation given in (2):

$$g = \frac{R\times\left({\pi rev.per\ minute}/{30}\right)^2}{Acceleration\ due\ to\ gravity} \tag{2}$$

where g = Decimal fraction of Earth gravity.

Plants grown in microgravity or simulated microgravity exhibit spontaneous auto morphogenesis (changes in growth direction), due to changes in plant hormones such as Auxin, Gibberellins and ethylene which serve as signal transducers responding to the changes in the gravity vector [5]. Different plants have unique responses to the changes in the gravity vector. Under microgravity conditions in space, the growth rates of many plant organs were reported to increase [6], but they were not changed or even decreased in some organs [7], [8].

In this study, *Lycopersicon esculentum* (Tomatoes) is subjected to a simulated microgravity condition in the laboratory for 72 hours which comprises germination and early growth. The growth rate and anatomical structure of the root tip was examined.

Gbenga F. Akomolafe is with the Department of Botany, Federal University Lafia, PMB 146, Lafia, Nigeria (phone: +2348068997606, e-mail: gfakomolafe@yahoo.com).

Joseph Omojola is with the Department of Physics, Federal University Lafia, Nigeria (e-mail: omojola.josef@gmail.com).

Seyi Adediwura, Ezekiel S. Joshua, and Michael O. Odey are with the Department of Physics, Federal University Lafia, Nigeria.

Elijah T. Adesuji and Ayo H. Labulo are with the Department of Chemistry, Federal University Lafia, Nigeria.

Oyinade A. Dedeke is with the Department of Plant Science and Biotechnlogy, Federal University Oye-Ekiti, Nigeria (e-mail: honeycrown1@gmail.com).

II. Materials and Methods

A. Collection of Seeds

Seeds of Tomatoes (*Lycopersicon esculentum)*: NG/MR/MAY/09/066 used for this study were collected from The National Centre for Genetic Research and Biotechnology (NACGRAB), Ibadan, Nigeria.

B. Growth Substrate Preparation and Germination of Seeds

A seed-supporting substrate for germination experiment called Agar-Agar was used. It is transparent for easier observation. It was prepared according to the standard method [9]. One hundred (100) ml of 1-1.5% Duchefa Biochemie Plant Agar-Agar in tap water (1.5 g agar-agar in 100 mL of tap water) was prepared. The agar-agar was boiled and stirred until no visible particles were left (up to two minutes) i.e. a clear solution. The solution was allowed to cool down to about 60°C. Three Petri dishes were filled with 10 ml to 25 ml of the agar-agar solution. The right depth of the agar-agar solution is such that the seeds can be embedded only halfway in the agar-agar, thus guaranteeing a supply of oxygen for the seeds. The agar-agar is allowed to cool down and solidify.

In each Petri dish, nine seeds of the tomatoes plant were planted on the agar-agar by using the tweezers in the same direction in order to identify the micropyle. After seeding the seeds on the agar-agar surface, two of the Petri dishes were placed vertically using a Petri dish holder as the control and the third Petri dish was mounted on the clinostat. The clinostat was rotated at a speed of 20 rpm for 72 hours (three days) inside a growth chamber. The set-up was isolated from light using a closed chamber keeping all environmental conditions equal for both the clinorotated and the control. The time of germination was recorded and germination percentage was calculated after germination.

After germination, one of the Petri dishes growing on normal gravity condition was rotated through 90 degrees to observe the response of the roots towards the gravity vector. The pictures of the three Petri dishes i.e. normal gravity (1 g), 90 degree rotated and clinorotated was taken at every 30 minutes using a Canon IXUS 160 digital camera (20 mega pixel) for three hours in order to determine the growth rate and root curvature.

C. Root Curvature Analysis

The root curvatures of the 90 degrees turned and clinorotated roots were determined following the standard methods [9]. It was done using an open-source image-processing application called ImageJ software. Out of the nine seedlings in each Petri dish, three uniformly germinated ones were selected for measurement and analysis at each time point i.e. every 30 minutes for three hours. Each result represents an average of three replicates.

D. Growth Rate Analysis

The growth rates were determined using standard methods [9]. Three uniformly germinated seedlings were selected for measurement and analysis measured using ImageJ software, from the nine seedlings in each Petri dish.

E. Anatomical Studies

After 72 hours, free-hand fresh transverse sections of the shoot and root of the clinorotated plant and normal gravity plant in water were made using a dissecting blade. Two or three drops of 1% Safranin O stain was transferred by pipette to a clean slide. The specimen was then placed by forceps to the drop of stain and left for one to two minutes. The stain was rinsed with three changes of distilled water. Hereafter, the stained specimens were dehydrated using ethanol. This was left for about one minute and rinsed with distilled water. The stained specimen were then transferred to a clean slide containing a drop of dilute glycerol and covered with a clean cover slip which is placed gently at an angle to avoid air bubbles. The cover slip was sealed with transparent nail polish. The mounted specimen was hereafter placed on the digital compound microscope for microscopic observation of its anatomical features. All quantitative data were subjected to the student's t-test between normal gravity (control) and clinorotated roots for significance difference.

III. Results

The seeds of this plant started germinating at 40 hours after planting. All environmental conditions were kept constant for both normal gravity and clinorotated (Table I). Both the normal gravity and clinorotated seeds started germinating at the same time. However, the percentage germination of normal gravity seeds was found to be (56%) higher than the clinorotated ones (44%). The clinorotated seeds did not germinate in a definite pattern. The orientation of the roots was in different directions, unlike the normal gravity sample which all germinated towards the direction of gravity (Fig. 1).

A. Root Curvature

The curvature angle of the 90 degree turned roots was far higher than the clinorotated ones at each time point (Fig. 2).

B. Growth Rate

The growth rate of the clinorotated roots and that of 1g increased with time after germination (Fig. 3). Also, at each time point after germination, the growth rate of the 1g was higher than clinorotated one.

C. Anatomical Studies

TABLE I
THE ENVIRONMENTAL VARIABLES AND GROWTH CONDITIONS OF NORMAL GRAVITY AND CLINOROTATED SEEDS

	Normal Gravity	Clinorotated
Relative Humidity at Planting	64%	64%
Relative Humidity at Germination	70%	70%
Temperature at Planting	26.1^0C	26.1^0C
Temperature at Germination	25^0C	25^0C
Percentage germination	56%	44%

It was observed that the normal gravity roots have distinct cellular arrangement unlike those of clinorotated. The roots cells of the normal gravity plants developed faster than the clinorotated as the boundaries between the cells were noticeable. No root hair was observed on the root of the

clinorotated plant. The thickness of the epidermis and cortex of the normal gravity root are higher than that of clinorotated (Table II). Also, the number of parenchyma cells per millimeter of the normal gravity plant was more than the clinorotated one.

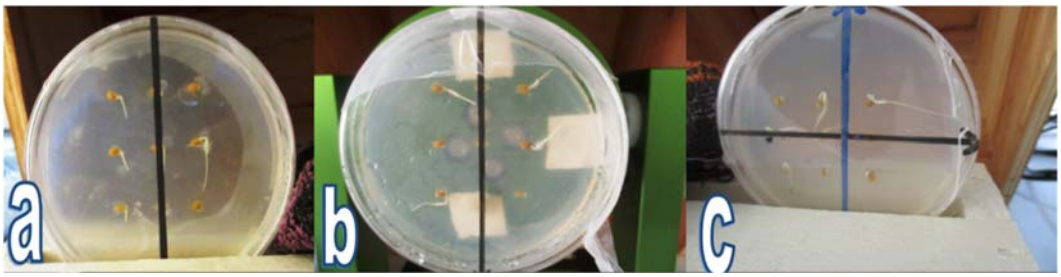

Fig. 1 (a) 1g *Lycopersicon esculentum* (b) clinorotated *Lycopersicon esculentum* (c) 90^0 rotated *Lycopersicon esculentum*

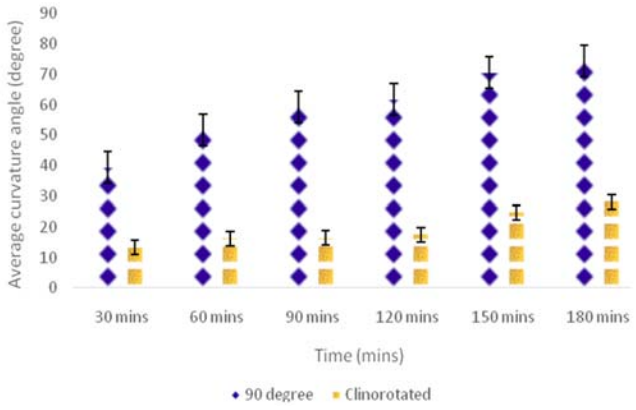

Fig. 2 The average curvature angle of the clinorotated roots and 90 degree turned roots of *Lycopersicon esculentum*

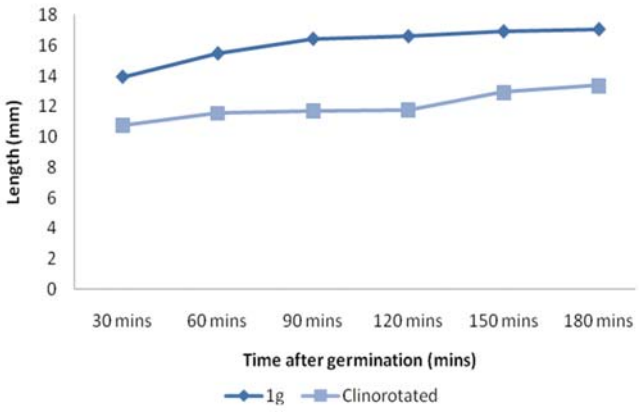

Fig. 3 The effect of clinorotation on the growth gate of *Lycopersicon esculentum*

TABLE II
THE ANATOMICAL FEATURES OF THE ROOT OF *LYCOPERSICON ESCULENTUM*

Anatomical features	Clinorotated	Normal Gravity
Length of root hairs	No root hairs observed	0.09mm - 0.13mm
Thickness of epidermis	0.01 ± 0.0 mm	0.02 ± 0.0 mm
Thickness of the cortex	0.30 ± 0.04 mm	0.36 ± 0.01 mm
Diameter of vascular bundle	0.22 ± 0.0 mm	0.24 ± 0.0 mm
No of cell per mm	20.0 ± 2.52 mm	22.33 ± 1.67 mm

Value represents mean ± SE and are significantly different at $\alpha \leq 0.05$

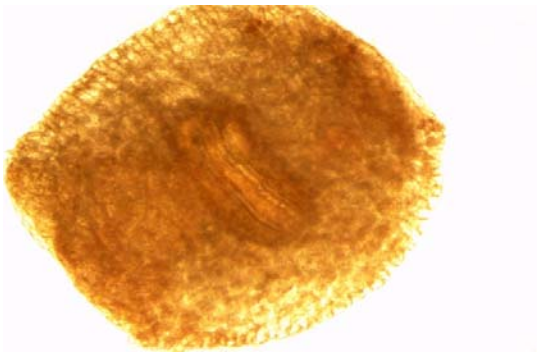

Fig. 4 The transverse section of the clinorotated root of *Lycopersicon esculentum*

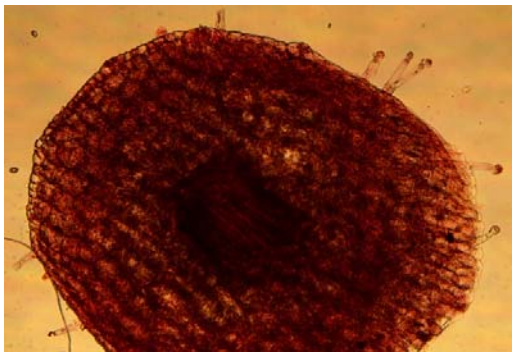

Fig. 5 The transverse section of the normal gravity root of *Lycopersicon esculentum*

IV. Discussion and Conclusion

The orientation of the clinorotated roots which were in different directions showed that the clinorotated roots could not sense gravity in any direction. And since there was no light influence, this could mean that the clinostat was able to create a simulated microgravity condition for the roots of this plant. The 90 degree turned roots were able to sense the direction of gravity, thereby leading to an increase in their root curvature angle unlike those of the clinorotated sample, which were under the influence of microgravity. Also, the higher growth rate observed in the normal gravity roots than the clinorotated could be because the growth hormones responsible for early growth were affected by microgravity. These noticeable differences

observed in the clinorotated roots could be attributed to the abiotic stress generated as a result of long duration of exposure of the roots to microgravity condition [5]. This is similar to [10] who observed fluctuations in the photosynthetic yield of some plants and attributed them to changes in gravity because series of parameters such as light intensity, temperature, pH, oxygen concentration, or obstruction of the measurements via air bubbles were kept constant. It also agrees with [11] who observed that photosynthetic functions such as the growth rate of wheat plants grown on space stations are affected by the microgravity environment.

The distinct cellular arrangement observed in normal gravity roots unlike that of clinorotated root, as well as reduction in the thickness of the epidermis and cortex is in agreement with [5] who reported that under long-term (days to months) microgravity exposure, plants acclimatize to the stress by changing their metabolism, as well as internal cellular features such as reduction the thickness of cells and rate of cell proliferation. These results conclusively show that microgravity can affect plants at the individual organ, tissue, cellular and sub-cellular levels.

ACKNOWLEDGMENT

We acknowledge the support received from The Nigerian Tertiary Education Trust Fund (TETFund) through Federal University Lafia Research and Linkages. We also acknowledge United Nations Office for Outer Space Affairs (UNOOSA) who donated the clinostat.

REFERENCES

[1] D. Moore and A. Cogoli. Biological and Medical Research in Space. Springer, 1996 ISBN-13:978-3-642-646-942.
[2] M. Fujie, H. kuroiwa, T. Suzuki and T. kuroia. Organelle DNA Synthesis in the quiescent centre of Arabidopsis thaliana. (col). J. Exp.Bot. vol. 44, 1993, pp. 690-693.
[3] H. Mirsandi, T. Yamamoto, Y. Takagi, Y. Okano, Y. Inatomi, Y. Hayakawa and S. Dost. A Numerical study on the growth process of InGaSb Crystal, 2015.
[4] J. Jing, C. Haiying and C. Weiming. Transcriptome Analysis of Oryza sativa Calli under Microgravity, vol. 27; 2015 pp. 437 – 453. Doi 10.1007/s12217-015-9432-2.
[5] H. Q. Zheng, F. Han and J. Le. Higher Plants in Space: Microgravity Perception, Response and Adaptation. Microgravity Science and Technology, vol. 27, 2015, pp. 377 – 386. Doi: 10.1007/s12217-015-9428-y
[6] T. W. Halstead and F. R. Dutcher. Plants in space. Annual Review of Plant Physiology vol. 38, 1987, pp. 317–345.
[7] J. Z. Kiss, W. J. Katembe and R. E. Edelmann. Gravitropism and development of wild-type and starch-deficient mutants of Arabidopsis during spaceflight. Physiology of Plant, vol. 102, 1998, pp. 493–502.
[8] L. H. Levine, A. G. Heyenga, H. G. Levine, J. W. Choi, L. B. Davin, A. D. Krikorian and N. G. Lewis. Cell-wall architecture and lignin composition of wheat developed in a microgravity environment. Phytochemistry, vol. 57, 2001, pp. 835– 846.
[9] United Nations Office for Outer Space Affairs (UNOOSA). Teacher's guide to plant experiments in microgravity- Human Space Technology Initiative. United Nations, New York, 2013.
[10] A. B. Simona, F. K. Matteo, E. S. Eva and V. A. Wilken-Jon. The Effects of Microgravity on the Photosynthetic Yield of Nannochloropsis Salina and Spirulina Platensis. International University Bremen, College Ring 6, 28759 Bremen, Germany, 2006.
[11] B. C. Tripathy, C. S. Brown, H. C. Levine and A. D. Krikorian. Growth and Photosynthetic Responses of Wheat Plants Grown in Space. Plant Physiology, vol. 110, 1996, pp. 801-806.