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GROWTH AND ANATOMICAL RESPONSES OF TOMATO *(LYCOPERSICON ESCULENTUM)* UNDER MICROGRAVITY AND NORMAL GRAVITY CONDITIONS

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ABSTRACT

Microgravity is known to be a major abiotic stress in space which affects plants depending on the duration of exposure. In this research, tomato 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 treatments. The orientation of the clinorotated roots during germination were in different directions unlike the normal gravity which all germinated towards the direction of gravity vector. The clinostat was able to switch off gravistimulation as distinct cellular arrangement was observed for the tomato plants under normal gravity condition unlike those of the clinorotated treatment. The results revealed that the thickness of the epidermis and cortex of the roots of normal gravity are higher than those of clinorotated. This suggests that under long-term microgravity exposure, plants can acclimatize to the stress by changing their internal cellular features such as reduction in the thickness of cells and rate of cell proliferation.

Keywords: *anatomy, clinostat, germination, microgravity, Lycopersicon esculentum*

INTRODUCTION

Microgravity is a characteristic of space environment, a condition of altered gravity which poses an abiotic stress on an organism's metabolism, growth and development (Jing *et al.*, 2015). Various platforms have been used to simulate microgravity condition. Examples include 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. International space station (ISS) which have been used as experimental platforms to conduct research in a true microgravity condition. Several authors have simulated plant's response to the condition of altered gravity (Moore *et al.*, 1987, Fujie *et al.*, 1993, Mirsandi *et al*., 2015, Jing *et al.*, 2015).

Gravity is a fundamental force that affects everything on Earth according to the relation given by Sir Isaac Newton in equation 1 below.

$$F = G\,{}^{M_1 M_2}/_{r^2} \quad \text{.....1}$$

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

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

Where g = Decimal fraction of Earth gravity

Plants grown in microgravity or simulated microgravity exhibit spontaneous auto morphogenesis (changes in growth direction) (Zheng *et al.*, 2015) due to changes in plant's hormones such as Auxin, Gibberellins and Ethylene which serve as signal transducers responding to the changes is the gravity vector. Different plants have unique responses to the changes in gravity vector. Under microgravity conditions in space, the growth rates of many plant organs were reported to increase (Halstead and Dutcher 1987), but they were not changed or even decreased in some organs (Kiss *et al.,* 1998, Levine *et al.,* 2001).

This study attempts to examine the growth rate and anatomical structure of the root tip of *Lycopersicon esculentum* under a simulated microgravity condition in the laboratory.

MATERIALS AND METHODS

Seeds of tomato (*Lycopersicon esculentum*), accession number NG/MR/MAY/09/066 which was used for this study was collected from the National Centre for Genetic Research and Biotechnology (NACGRAB), Ibadan, Nigeria.

Agar-agar was used as a seed-supporting substrate for germination experiment. The substrate which is transparent for clear observation was prepared according to standard method (UNOOSA, 2013). 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 are left (up to two minutes) to obtain 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 tomato plants 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 control and the third petri dish was mounted on the clinostat. The clinostat was rotated at a speed of 20 rpm for 72 hours (3 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 gravity vector. The pictures of the 3 Petri dishes i.e. normal gravity (1g), 90 degree rotated and clinorotated was taken at every 30 minutes using a Canon ixus 160 digital camera (20 mega pixel) for 3 hours in order to determine the growth rate and root curvature.

The root curvatures of the 90 degrees turned and clinorotated roots were determined following the standard methods (UNOOSA, 2013). 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 3 hours. Each result represents an average of three replicates.

The growth rates were determined using standard methods (UNOOSA, 2013). Out of the nine seedlings in each petri dish, three uniformly germinated ones were selected for measurement and analysis measured using ImageJ software.

The anatomical studies commenced after 72 hours, when free-hand fresh transverse sections of the shoot and root of the clinorotated plant and normal gravity plant in water were prepared 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 1 to 2 minutes. The stain was rinsed with 3 changes of distilled water. Hereafter, the stained specimens were dehydrated using ethanol. This was left for 1 minute and then rinsed with distilled water. The stained specimens 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 student's t-test between the normal gravity (control) and clinorotated roots for significance difference.

RESULTS AND DISCUSSION

The seeds of the plant started germinating at 40 hours after planting. All environmental conditions were kept constant for both normal gravity and clinorotated (Table 1). 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 definite pattern. The orientation of the roots was in different directions unlike the normal gravity which all germinated towards the direction of gravity (Fig. 1). The curvature angle of the 90 degree turned roots was far higher than the clinorotated ones at each time point (Fig. 2). 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.

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 increase in their root curvature angle unlike those of clinorotated 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 clinorotated roots could be attributed to the abiotic stress generated as a result of long duration of exposure of the roots to microgravity condition (Zheng *et al*., 2015). This is similar to Simona *et al*., (2006) 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 Tripathy (1996) who observed that photosynthetic functions such as growth rate of wheat plants grown on space stations are affected by the microgravity environment.

It was observed that the normal gravity roots have distinct cellular arrangement unlike those of clinorotated. The roots cells of normal gravity plants developed faster than 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 2). Also, the number of parenchyma cells per millimeter of normal gravity plant was more than the clinorotated one. These observations are in agreement with Zheng *et al*., (2015) who reported that under long-term (days to months) microgravity exposure, plants acclimatize to the stress by changing their metabolism, internal cellular features such as reduction in the thickness of cells and rate of cell proliferation.

CONCLUSION

These results conclusively show that microgravity can affect plants at the individual organ, tissue, cellular and sub-cellular levels.

Table 1: 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	26.10C	26.10C
Temperature at Germination	250C	250C
Percentage germination	56%	44%

Table 2: 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 $\alpha \leq 0.05$

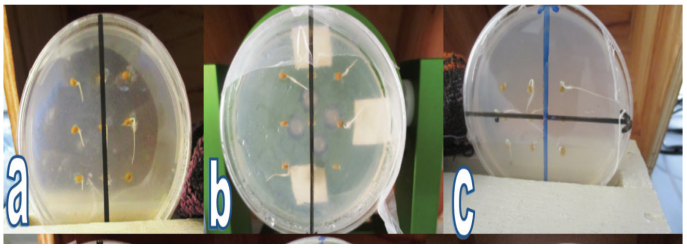

Fig. 1:(a) 1g *Lycopersicon esculentum* (b) clinorotated *Lycopersicon esculentum* (c) 900 rotated *Lycopersicon esculentum*

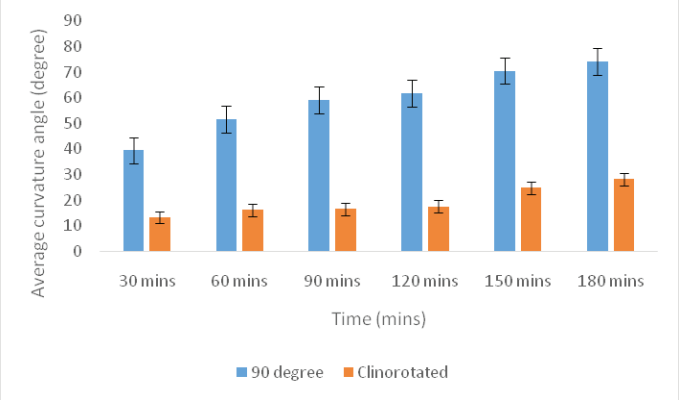

Fig. 2: The average curvature angle of the clinorotated roots and 90 degree turned roots of *Lycopersicon esculentum*

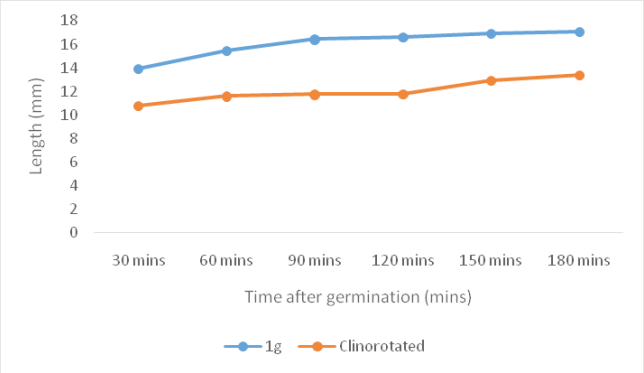

Fig. 3: The effect of clinorotation on the growth gate of *Lycopersicon esculentum*

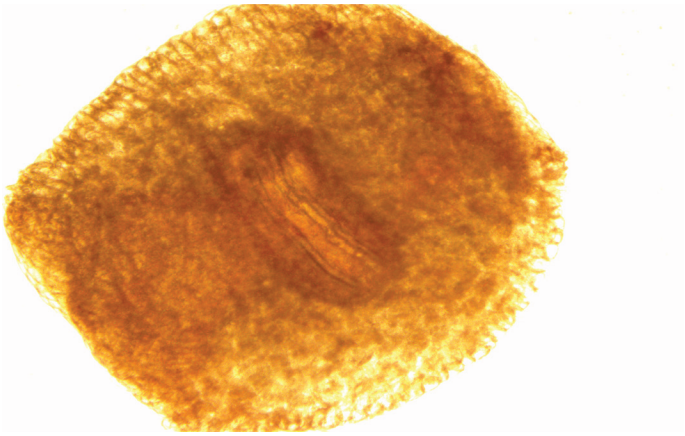

Fig. 4: The transverse section of the clinorotated root of *Lycopersicon esculentum*

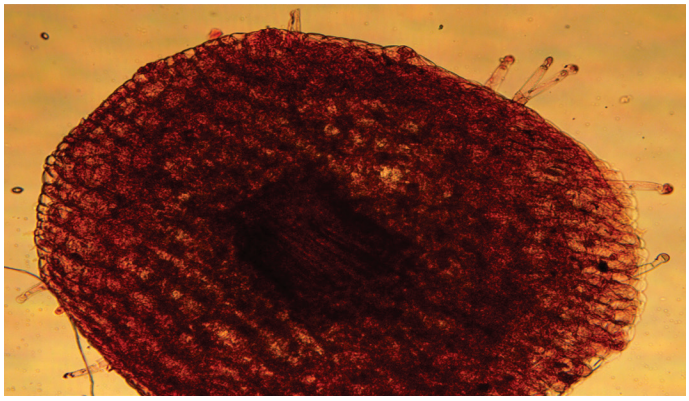

Fig. 5: The transverse section of the normal gravity root of *Lycopersicon esculentum*

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REFERENCES

Cowls, J. R, Scheld, H. W, Lemay, R., Peterson, C. (1984). Growth and lignifications in seed lings exposed to eight days of microgravity. *Annals of Botany*

Fujie, M., kuroiwa, H., Suzuki, T, kuroia, T. (1993). Organelle DNA Synthesis in the quiescent centre of Arabidopsis thaliana. (col). *J. Exp.Bot*. 44, 690-693.

Hader, D.P, Hemmersbach, R, & Lebert, M. (2005). Gravity and the behaviour of Unicellular organisms. Cambridge University Press Page 14 & 30.

Halstead, T.W. and Dutcher, F. R. (1987). Plants in space. Annual Review of Plant Physiology 38: 317–345.

Hoson, T (2014). Plant Growth and Morphogenesis under Different Gravity Condition: Relevance to Plant Life in Space. *Life* 4, 205 – 216; doi:10.3390/life4020205

Hu, L.. Haiying, C., Xianying, D., Jing, J, Weiming, S and Weiming, C. (2015). Proteomic Analysis of the upper and lower flanks of the Base of Rice Shoot in the Gravitropism. *Microgravity Science and Technology,* 27: 403 – 416. Doi: 10.1007/s12217-015-9433-1

Jing, J. Haiying, C. and Weiming, C. (2015). Transcriptome Analysis of *Oryza sativa Calli* under Microgravity 27; 437 – 453. Doi 10.1007/s12217-015-9432-2.

Kiss, J. Z., Katembe, W. J. and Edelmann, R. E. (1998). Gravitropism and development of wild-type and starch-deficient mutants of Arabidopsis during spaceflight. *Physiology of Plant* 102: 493–502.

Levine, L. H., Heyenga, A. G., Levine, H. G., Choi, J. W., Davin, L. B., Krikorian, A. D. and Lewis, N. G. (2001). Cell-wall architecture and lignin composition of wheat developed in a environment. *Phytochemistry* 57: 835– 846.

Mirsandi, H., Yamamoto, T., Takagi, Y. Okano, Y., Inatomi, Y., Hayakawa, Y., and Dost, S. (2015). A Numerical study on the growth process of InGaSb Crystal

Moore, D., and Cogoli, A. (1996). Biological and Medical Research in Space. Springer, ISBN-13:978-3-642-646-942.

Simona, A. B., Matteo, F. K., Eva, E. S. and Wilken-Jon, V. A. (2006). The Effects of Microgravity on the Photosynthetic Yield of *Nannochloropsis Salina and Spirulina Platensis*. International University Bremen, College Ring 6, 28759 Bremen, Germany.

Tripathy, B. C., Brown, C. S., Levine, H. C., and Krikorian, A. D. (1996). Growth and Photosynthetic Responses of Wheat Plants Grown in Space. *Plant Physiology* 110: 801- 806, United Nations Office for Outer Space Affairs (UNOOSA) (2013).

UNOOSA (2013). Teacher's guide to plant experiments in microgravity- Human Space Technology Initiative. United Nations, New York.

Zheng, H. Q., Han, F., and Le, J. (2015). Higher Plants in Space: Microgravity Perception, Response and Adaptation. *Microgravity Science and Technology*, 27: 377 – 386. Doi: 10.1007/s12217-015-9428-y