Plant Cell Biotechnology and Molecular Biology 21(43&44):92-104; 2020 ISSN: 0972-2025

CLINOSTAT MICROGRAVITY IMPACT ON ROOT MORPHOLOGY OF SELECTED NUTRITIONAL AND ECONOMIC CROPS

FUNMILOLA ADEBISI OLUWAFEMI, OMODELE IBRAHEEM[*] AND TOLUWASE HEZEKIAH FATOKI

Astrobiology Unit, Space Life Sciences Division, Department of Engineering and Space Systems, National Space Research and Development Agency (NASRDA), Airport Road, Abuja, Nigeria [FAO].
Plants for Biotechnological Resources Research Group, Department of Biochemistry, Federal University Oye-Ekiti, P.M.B. 373, Oye-Ekiti, Ekiti State, Nigeria [FAO, OI, THF].
[*For Correspondence: E-mail: deleibraheem2007@yahoo.com]

Article Information

Editor(s):
(1) Dr. Ravi Kant Chaturvedi, Chinese Academy of Sciences, P. R. China.
Reviewers:
(1) Hanan Omar Badr Eldin, National Research Center, Egypt.
(2) Lindomar Maria de Souza, Universidade Federal Rural de Pernambuco, Brazil.

Received: 10 July 2020
Accepted: 14 September 2020
Published: 09 October 2020

Original Research Article

ABSTRACT

The use of the microgravity simulators such as Clinostat has enhanced microgravity research on Earth as engaging in real space flight microgravity experiments are expensive and unusual. The reduction of gravity causes significant changes on biological organisms, macromolecules, fluids and materials. Some of these changes that occur have led to discoveries that have been found to be of social-economic benefits. The use of Clinostat as a source of simulated microgravity impact for the growth of plants has produced several positive implications especially for the agriculture sector. It is also predicted that space explorers in distant future will have improved breeds of crops that will adapt and survive well in the harsh environment of space from microgravity simulations experiments from Earth. In this research, 10 essential plants: peanut, cowpea, watermelon, okra, cotton, cucumber, wheat, sorghum, rice and corn were selected because of their nutritional and economical values. They were separately grown under normal Earth gravity (control) and under simulated-microgravity (clinorotation) using Clinostat. The experimental variables on the Clinostat were rotation-speed, rotational-axis angle and rotation-direction. Observations were made for hours during the experiment on the roots morphological developments range from the physical characterization of the roots to the growth-rate and root-curvature using ImageJ software. Results revealed that there were mostly improved growth-rates and reduced response to gravity per-hour on the microgravity simulated samples than the control plants. The clinorotated-samples root-curvatures ranged from 1.33°/hr to 28.25°/hr for the ten plants; while the 90°-turned sample ranged from 3.99°/hr to 33.13°/hr. The clinorotated-samples of eight plants showed increased growth-rate per hour than their 1 g-control and ranged from 2 mm/hr to 10.75 mm/hr while cotton and sorghum

had 2.13 mm/hr and 6.08 mm/hr respectively as decrease in growth-rates. The growth-rate for 1 g-control of the ten plants ranged from 0.69 mm/hr to 8.01 mm/hr.

Keywords: Plants; root morphology; clinostat; simulated microgravity.

INTRODUCTION

Microgravity is a major characteristic of the space environment. Several researches under real microgravity have been carried out on plant growth using space flights, one of such is the one performed on the International Space Station (ISS) called Advanced Plant Experiments (APEX) [1]. Space flight microgravity experiments are scarce and expensive, therefore restricting several researchers in this area. Therefore, a substitute ground-based facility, Clinostat was considered [2].

A Clinostat is an experimental instrument used in a laboratory on the Earth to simulate (mimic) microgravity or to remove the effect of gravity. There are three types, 1D, 2D and 3D. A 2D Clinostat is a one axis Clinostat while 3D Clinostat is a two axis Clinostat which is also called a Random Positioning Machine (RPM). The Clinostat used in this research is a desk-top one axis (2D) Clinostat. A one axis Clinostat is a two dimensional Clinostat having a single rotational axis that runs perpendicular to direction of the gravity vector [2,3]. It is operational in respect to the direction of rotation and speed. On a Clinostat, a rotation is named ‘clinorotation’. One axis Clinostat has been used in the field of plant physiology.

The Clinostat used in this research was given to National Space Research and Development Agency (NASRDA), Abuja, Nigeria by United Nations Office for Outer Space Affairs (UNOOSA) in the ‘Zero Gravity Instrument Project’ (ZGIP), 2015 for educational and research purposes.

The Clinostat is used to ascertain how biological organisms and matter respond when gravity is removed. Possible samples useable on it are: Biological organisms (plants, humans, cells, micro-organisms), fluids, macromolecules and materials. In this study, fast clinorotation effect on the growth of 10 essential economic plants was determined. Fast clinorotation – fast rotation speed is necessary for research purposes. The selected plants were either monocotyledon or dicotyledon; and either legume, vegetable, fruit or grain. These plants are peanut, cowpea, watermelon, okra, cotton, cucumber, wheat, sorghum, rice and corn. Among the plants, cowpea, peanut, watermelon, okra, cucumber and cotton are dicot while wheat, sorghum, corn and rice are monocot. Cowpea and peanut are legumes; cucumber is a vegetable; watermelon and okra are fruits; while cotton, wheat, sorghum, corn and rice are grains. The novelty of this work is in the impartation of microgravity simulations using the research equipment – Clinostat on these plants, as it is a scarce research equipment.

These plants were chosen majorly because of their economic and nutritional content and uses to human. The other properties that made them suitable for this research are that their seeds are small, fast growing, easy to handle with germination period at a maximum of 4 days. These properties made the seeds also useable on the Clinostat as the 2D Clinostat used in this research does not take more than 500 g weight of sample. Plants roots are specialized for absorption, anchorage, conduction and even storage [4]. This technique could be of immense benefit to agriculture as it could activate the seedlings innate cascade mechanisms that could positively affect their inbuilt architecture for higher productivity, nutritional qualities and resistance to environmental stresses when transplanted unto the field.

Thus, this type of systematic research under microgravity will offer insights into plants developments and understanding their responses to gravity and microgravity can lead to novel applications that will be advantageous to man in terms of food security, medical treatments etc. and will also generate experimental data-sets in microgravity responses, which will contribute greatly to the designing of imminent space experiments and to the progress of microgravity research [2].

Many researches have shown that most of the time the impact of simulated microgravity on plants are positive on the plant biochemicals [5,6], therefore affecting their growth and development [7]. Simulated microgravity has also been a very useful tool in understanding the growth and development of plants in altered gravity conditions in order to observe the changes in each growth process under a weightless environment [8,9]. Report by Oluwafemi and Olubiyi [10] indicated a better growth-rate of corn under simulated microgravity using Clinostat.

Plants roots are structures specialized to function for anchorage, storage of photoassimilates, absorption and conduction of water and minerals from soil, which make them very important for gravity response and in plant physiology matters [4]. From the aforesaid, this research focuses on the morphological developments of plant roots. Genetics and biochemical make-up play major roles during rooting process in plants and subsequently the overall development of the plant. Thus, root determines the quality and quantity of fruits, without root no fruits, therefore studying roots morphological system will provide information on other fundamental plant developmental processes, as a whole plant starts its growth firstly from a seed by the development of the roots, later shoot development [9].

Thus, the objectives of this research work were to: determine the impact of gravity on the sample plants; ascertain their orientation in simulated microgravity; identify the fundamental mechanisms; and make observations by comparing the differences in the germination of plants under control Earth's surface gravity (1 g) experiments to simulated microgravity experiments.

MATERIALS AND METHODS

Seeds of ten different plant species (peanut, cowpea, watermelon, okra, cotton, cucumber, wheat, sorghum, rice and corn) were purchased at the International Institute of Tropical Agriculture (IITA), Kubwa, Abuja, Nigeria and were also authenticated to be the genuine seeds sought after. Seeds of each species (per experiment) were planted into 3 petri dishes (9 seeds per petri dish; three seeds in parallels) using plant substrate, agar-agar, using the preparation standard method in the UNOOSA Teacher's Guide to Plant Experiments [2]. The gel strength of the Duchefa Biochemie agar-agar (Netherlands) used was >1100 g/cm^2 (Prod. No.: P1001.1000; Lot. No.: B010857.07; CAS No.:9002-18-0).

A petri dish was placed on the template for seed placement (Fig. 1) with the back side facing towards the experimenter. Using a permanent marker, a vertical reference line was drawn on each of the 3 petri dishes to be used; and a mark was put at the top of the line. This line indicates the direction of the gravity vector. The mark at the top indicates the up side of the petri dish.

100 ml of 1.5% Duchefa Biochemie plant agar-agar was prepared in water (1.5 g plant agar-agar in 100 ml of water). Well water was used. This is because the experiment is been done to be as close as possible to the real farm setting; as on the farm, distilled water will not be used. The well water used has acceptable qualitative water analysis assessment for plant cultivation according to UMassAmherst [11].

The agar-agar was boiled until no visible particles were left (up to two minutes). The solution was clear and stirred well. The solution was allowed to cool down for about 5 minutes to about 60°C. Agar-agar becomes solid at a temperature lower than 37°C.

The 3 petri dishes were filled with 10 ml to 25 ml (depending on the chosen seeds) of the agar-agar solution. The right depth of the agar-agar solution is such that the seed can be embedded only halfway in the agar-agar, thus guaranteeing a supply of oxygen for the seeds. The petri dishes were then covered with the lid. It is important that the complete bottom surface of the petri dishes be covered homogenously with agar-agar, so it was made sure that the filled petri dishes were turned slightly on the table when pouring the agar-agar.

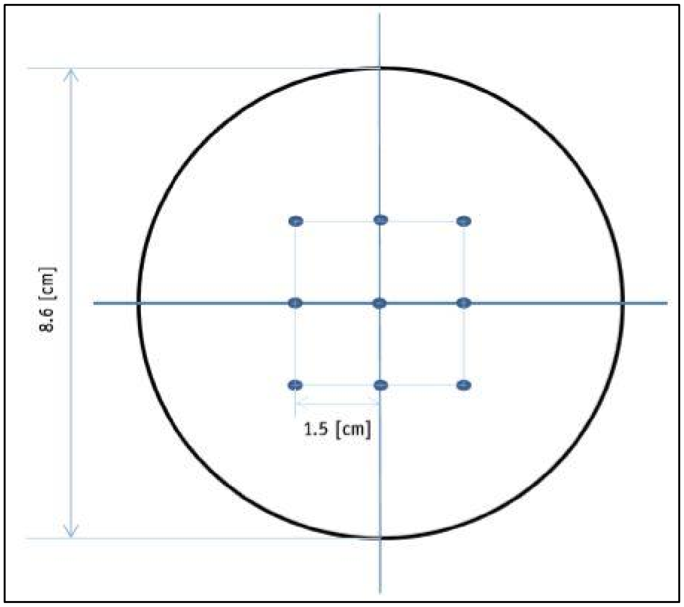

Fig. 1. Template for seed placement

The agar-agar was allowed to become slightly solid. This took from 5 to 10 minutes. After the agar-agar cools down, if there is condensation of water on the underside of the lid, it was removed by tapping the lid. Each petri dish was put on the template for seed placement. In each petri dish, was placed 9 seeds on the agar-agar by using the tweezers. All seeds were planted in the same direction using the black line (micropyle) on each seed in order to orient it. The lids were then used to close the petri dishes. After seeding on the agar-agar surface, the seeds took up water in the outer cell layer. After this stage, the petri dishes were then rotated vertically.

After it is made sure that the seeds are stuck on the agar-agar surface, parafilm or transparent sticky tape was used to affix each lid onto the petri dishes. Some gaps were put between the lid and the dish for proper oxygen support. The petri dishes were placed in vertical positions using wooden holder to support the petri dishes. It was made sure that the vertical reference line on each petri dish was made parallel to the gravity vector (i.e. they were held in alignment with the direction of the gravity vector). A wet chamber was prepared in a plastic box with a size of about 40 cm x 40 cm x 40 cm whereby 5 liters of water was put into it. The following conditions were sustained throughout the experiment: Temperature of 25°C, light of 50 lux, and humidity 80%.

After few days under Earth's gravity, the seeds germination with short roots (about 50 mm) was noticed. The 3 petri dishes were thereafter labeled 1 g-control, 90º-turned and Clinorotated. The 1 g-control sample was left in its vertical position. The 90º-turned sample was rotated by 90 degree (anticlockwise), while the clinorotated-sample was placed on the 2D Clinostat (manufactured by Advanced Engineering Services., Co. Ltd. Model UN-KTM2 REV. NC. 2012.11) (see Fig. 2) by using the double-sided tape. Therefore, the 1 g-control and the 90º-turned samples were still under Earth's gravity, while the clinorotated-sample on the Clinostat was under simulated microgravity. The 1 g-control sample and the 90º-turned sample served as control experiments for growth-rate and root-curvature analyzes respectively. The 90º-turned samples were able to authenticate that gravity was active in the environment used for experiment. The clinorotated-sample on the Clinostat was under these subsequent conditions: clockwise rotation direction, fast rotation speed, and rotational axis angle of 90º. Table 1 shows the specifications (key parameters) of the 2D Clinostat.

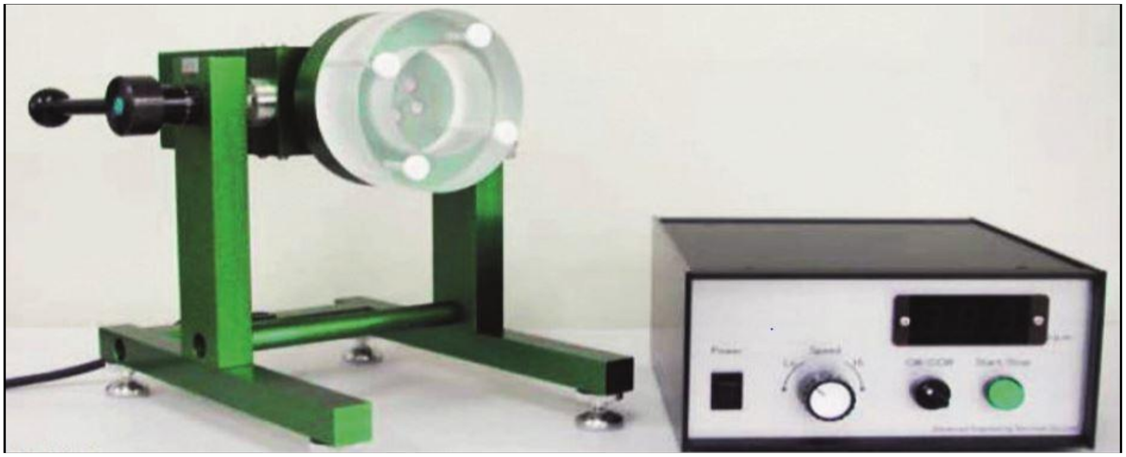

Fig. 2. One axis clinostat with its control box in its horizontal rotation position

Table 1. Specifications of the 2D Clinostat

Rotational speed	0-90 rpm 0-20 rpm: 0.5 rpm increments 20-90 rpm: 5 rpm increments
Rotational axis angle	0° (parallel to the ground) to 90° (perpendicular to the ground)
Rotation direction	Clockwise or counterclockwise
Experiment conditions	Maximum weight of samples: 500 g Maximum diameter of a sample container: 10 cm

Table 2. Parameters employed for the germination and growth of the 10 plants

Seed name	Germination period (days)	Duration of rotation on clinostat (hr)	Rotational speed (rpm)	Light (lux)
Cowpea (*Vigna unguiculata*)	3	6½	80	45
Peanut (*Arachis hypogae*)	2	2	15	50
Wheat (*Triticum*)	2	6	75	50
Okra (*Abelmoschus esculentus*)	2	6	80	50
Cucumber (*Cucumis sativus*)	2	5	80	50
Watermelon (*Citrullus lanatus*)	4	2	80	50
Cotton (*Gossypium*)	3	4	85	50
Sorghum (*Sorghum bicolor*)	3	4	85	50
Corn (*Zea mays*)	3	2	80	50
Rice (*Oryza sativa*)	3	2	75	50

The images of the 3 petri dishes were taken using Canon A300 Digital Camera of 10 Megapixels Sensor with 3.3X Optical Zoom Lens at 30 minutes interval. The clinorotated-sample was stopped for just few seconds to take its image in order to prevent the effect of gravity. The observations on these samples were done varying the time of the experiment (between 2 to 6 hours) and the rotation speed. Note that the temperature, light conditions, humidity, rotation direction, rotation speed, rotational axis angle (horizontal or vertical), and the time of observation are the possible variables of the experiment. In this research, temperature, light conditions, humidity, rotation direction (Table 2) and rotational axis angle (horizontal) (Fig. 2) were kept constant while the time of observation was varied for the plants. The rationale used to mount the clinorotated-sample on the Clinostat was that the root should germinate to 50 mm for optimal results.

After observations, the plants root morphology was studied using ImageJ software to analyze the roots angles and roots lengths from the sets of images taken. The root-curvature and growth-rates analyzes of the selected plants were determined by using their root angles and root lengths respectively under 1 g controlled environment and under simulated microgravity and the results were compared. The grand average root angles and root lengths of the seeds per plant were calculated per hour resulting in the root-curvatures and growth-rates respectively.

Data was initially tabulated on Microsoft Excel 2010 and GraphPad Prism v6.0; and later transferred to the Statistical Package for Social Sciences version 22.0 (SPSS Inc., Chicago, IL, USA). A One-way Analysis of Variance (ANOVA) was used for the analysis. The Least Significant Difference (LSD) was used as post hoc test. Statistical significance levels were set at 5% ($P < 0.05$).

RESULTS AND DISCUSSION

The obtained data were the sets of images obtained using Canon A300 Digital Camera of 10 Megapixels Sensor with 3.3X Optical Zoom Lens of the roots for clinorotated, 1 g-control and 90°-turned plants treatments. The image processing application specialized software called ImageJ was used to analyze these images (Fig. 3). The images in Fig. 3 were taken 30 minutes to the end of the observing period. The roots of the 90°-turned sample images (as seen from Fig. 3) are facing towards right because they were turned 90°.

Root-curvature

The root-curvature focuses on the curvature of the roots on the images of the 90º-turned sample and the clinorotated-sample. All the root angles were measured using the tool for angle measurement on the ImageJ. After the curvature angles were gotten, the real curvature angles were calculated by subtracting the known measured angle from 180°. The average angular rate of the root in degrees (per hour) was then calculated.

The images of the 90°-turned samples of the plants per experiment indicated that the roots started bending vertically in the direction of gravity after the petri dishes were turned by 90 degree (see Fig. 3). The bending of the 90º-turned sample was more than that of the clinorotated-roots. The direction of the roots of the clinorotated-samples was haphazardly arranged following no pattern. Its shown in the results that the clinorotated-samples of all the 10 plants had reduced response to gravity (per hour) when compared to the 90°-turned samples.

The average value of all the root angles of the nine seeds on the 90°-turned sample for each time points was calculated, afterwards, the grand average of the roots was then calculated. This grand average value was divided by the duration (in hr) of observation. The clinorotated-samples of all the 10 plants had reduced response to gravity per hour when compared to their 90°-turned counterparts. The root-curvatures of the clinorotated-samples range from 1.33°/hr to 28.25°/hr for the 10 plants while the 90°-turned sample range from 3.99°/hr to 33.13°/hr. The summaries of the rate of root-curvature data and the statistics are as presented in Table 3 and Fig. 4.

It was observed that the 1g-control samples showed that the roots grew continuously vertically as stimulated by the Earth's gravity (see Fig. 3). For the clinorotated-roots, nothing stimulated their growth in any specific direction i.e. their growth was random. This was an indication of gravitropism of the roots. Since there was a decrease in the average angular rates of the roots bending for the 90°-turned samples compared to the clinorotated, it means that the clinorotated-samples showed positive responses to simulated microgravity.

Root Growth-rate

The 1 g-control and the clinorotated-roots images taken of all the plants per experiment were used to investigate the growth-rate of the seeds of the sample plants. This was carried out by measuring the roots length, which allowed for the determination of their growth-rates. For corn and sorghum that had two or three roots per seed, the longest of the roots was measured, while the single root of all the other plant species was measured. The length of the roots was determined by drawing a line which is precisely 10 mm on each petri dish. The line was normalized to 10 mm length on the ImageJ software which acts as a fixed length in the photo. After the normalization, the length measurement tool of the ImageJ software was used to measure the length of each of the root [3].

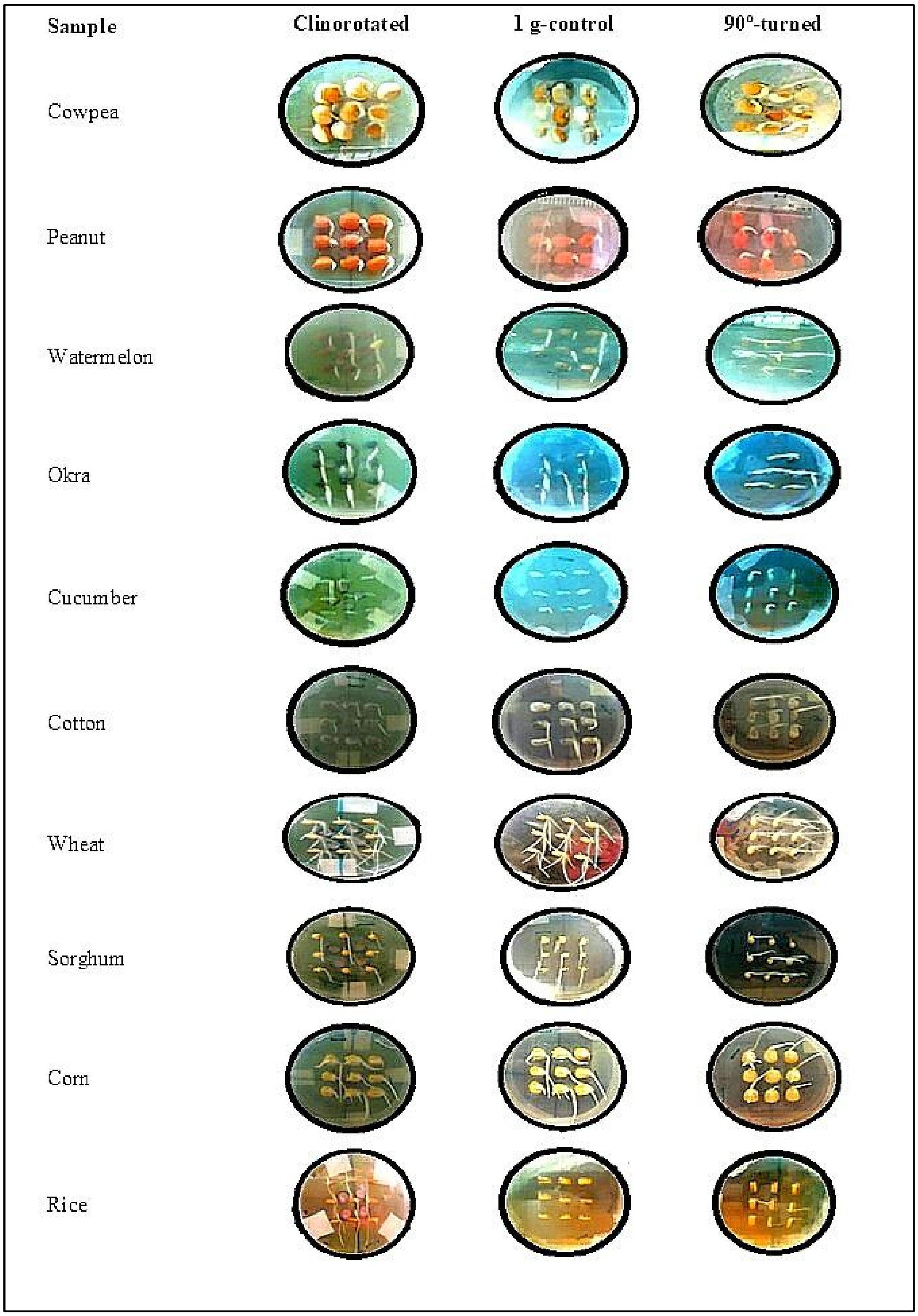

Fig. 3. Sets of images of the roots of cowpea; peanut; watermelon; okra; cucumber; cotton; wheat; sorghum; corn; and rice. The clinorotated; 1 g-control; and 90°-turned, represent for the plants treatments

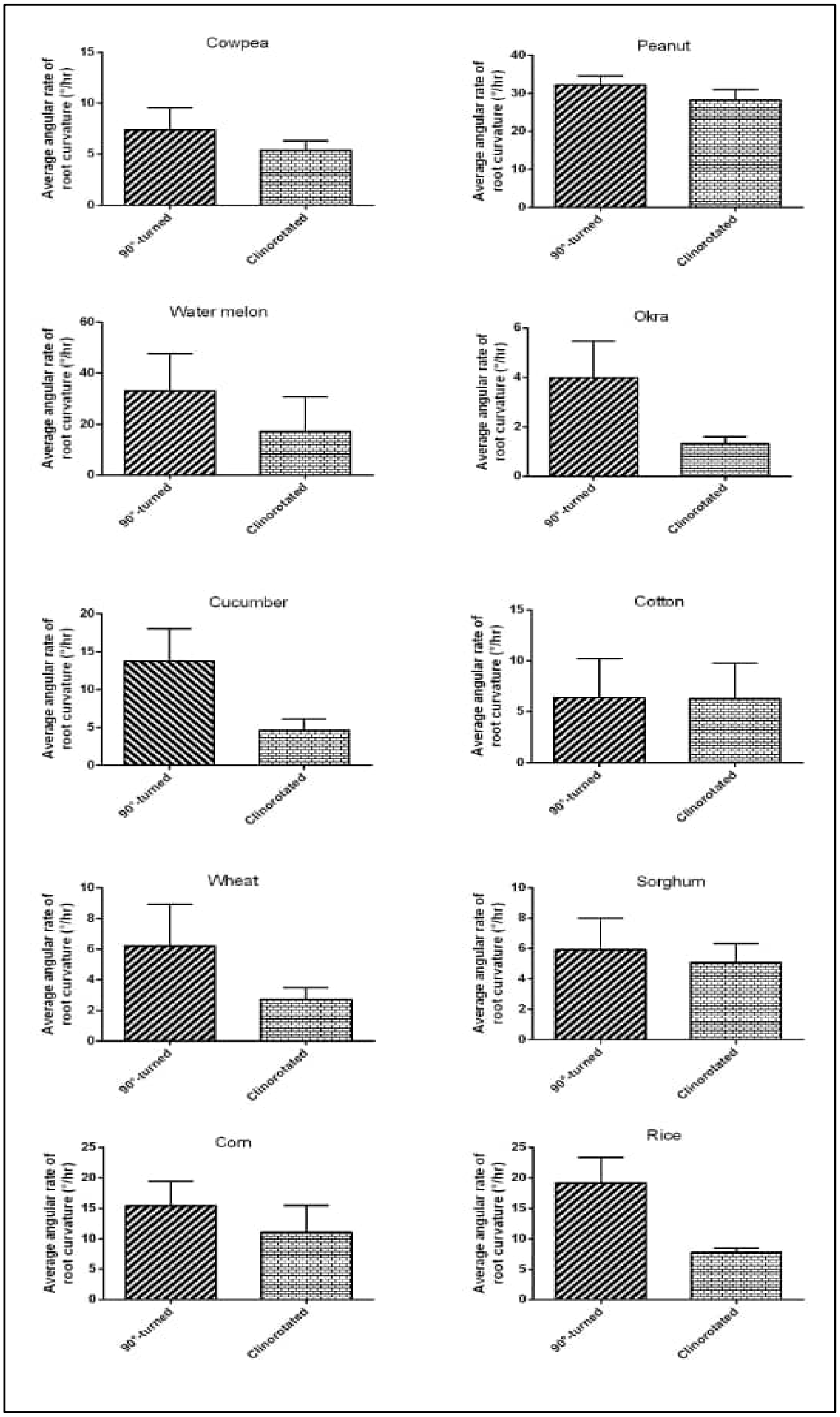

Fig. 4. Sets of bar charts for the grand average rate root-curvature of cowpea; peanut; watermelon; okra; cucumber; cotton; wheat; sorghum; corn; and rice for 90°-turned and clinorotated plants treatments

Table 3. Shows the grand average rate of root-curvature of the selected plants (n=9)

Plant	90°-turned Mean (°/hr)	90°-turned Standard deviation (°/hr)	Clinorotated Mean (°/hr)	Clinorotated Standard deviation (°/hr)
Cowpea	7.40	2.16	5.40	0.90
Peanut	32.25	2.38	28.25	2.78
Wheat	6.20	2.73	2.72	0.77
Okra	3.99	1.47	1.33	0.28
Cucumber	13.78	4.22	4.62	1.50
Watermelon	33.13	14.58	17.18	13.64
Cotton	6.39	3.84	6.30	3.45
Sorghum	5.91	2.07	5.09	1.22
Corn	15.43	3.99	11.09	4.40
Rice	19.14	4.20	7.74	0.75

The average value of all the length of the nine 1 g-roots for each time points was evaluated and thereafter, the grand average of the lengths was calculated. This grand average value was divided by the duration (in hr) of observation. It was observed that the clinorotated-samples of eight plants except cotton and sorghum showed an increase in growth-rate per hour than the counterpart 1 g-control samples. The growth-rates of the clinorotated-samples of these eight plants, range from 2 mm/hr to 10.75 mm/hr while cotton and sorghum had 2.13 mm/hr and 6.08 mm/hr respectively as reduced growth-rates. The growth-rate for 1g-control of all the ten plants range from 0.69 mm/hr to 8.01 mm/hr. Fig. 5 shows the grand average growth-rate of the selected plants. The percent decrease in root-curvature of the clinorotated compared to 90°-turned of the samples, and percent increase including the statistics in the growth-rate of the clinorotated compared to the 1g-control of the samples is seen on Table 4 and Fig. 6.

The average growth-rate of the roots of the plants except cotton and sorghum were increased under simulated microgravity. It is also evident from Fig. 4 that the average angular rate of root-curvatures of the clinorotated-samples of cotton and sorghum, though was decreased compared to 90°-turned, but are very close. This can support the reduction in their growth-rates.

To experiment on the effects of the environment of spaceflight on plants, a series of Advanced Plant Experiments (APEX) was done on Veggie facility onboard the ISS. Plants were researched on to discover the novel pathways that cause the complex cellular processes shaped by gravity on plant development [1]. Many of the result obtained in this research were also in conformity with several research that have been done as enumerated in the next paragraphs.

Table 4. The grand average growth-rate of the root of the selected plant (n=9)

Plant	90°-turned Mean (mm/hr)	90°-turned Standard deviation (mm/hr)	Clinorotated Mean (mm/hr)	Clinorotated Standard deviation (mm/hr)
Cowpea	1.73	0.36	3.28	0.44
Peanut	4.20	0.12	5.45	0.77
Wheat	4.89	0.55	5.16	0.93
Okra	3.07	0.30	3.50	0.50
Cucumber	0.69	0.09	2.00	0.73
Watermelon	4.94	1.11	6.58	0.53
Cotton	3.21	0.50	2.13	0.43
Sorghum	8.01	3.23	6.08	2.34
Corn	7.54	0.52	9.73	0.46
Rice	5.26	0.47	10.75	1.60

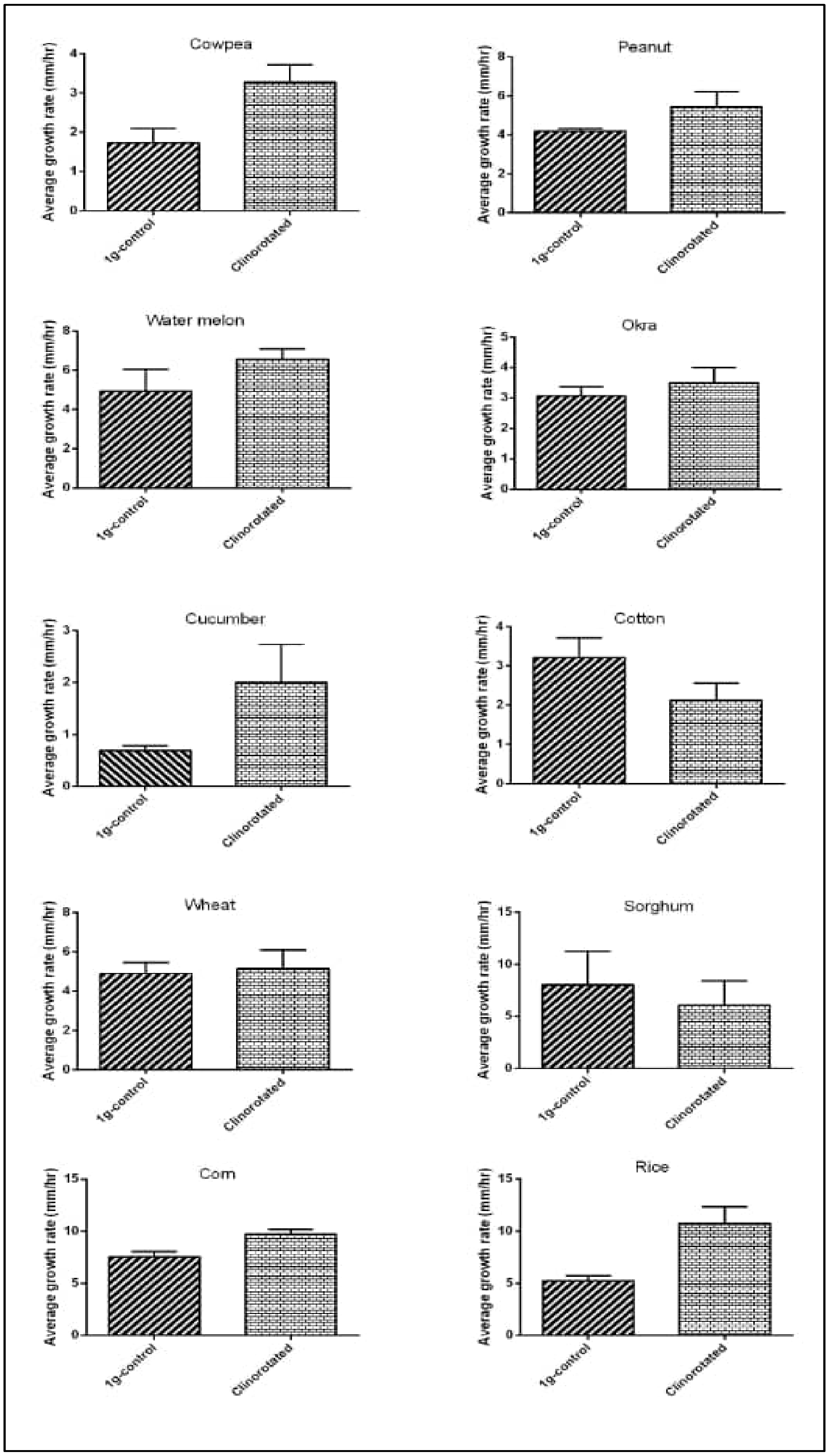

Fig. 5. Sets of bar charts for the grand average root growth-rate of cowpea; peanut; watermelon; okra; cucumber; cotton; wheat; sorghum; corn; and rice for 1 g-control and clinorotated plants treatments

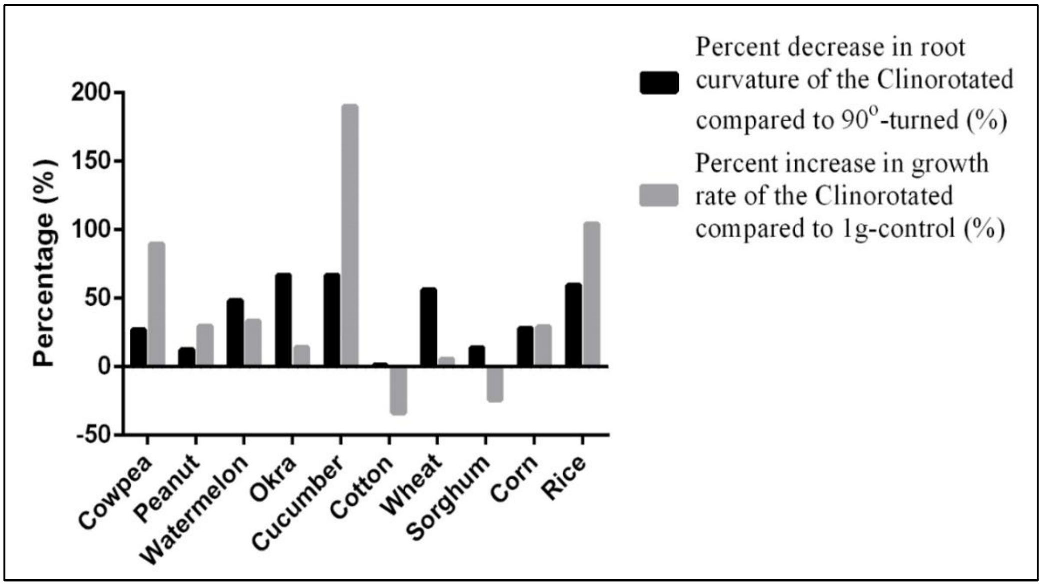

Fig. 6. Percentage change of the clinorotated treatment compared to 90°-turned and 1 g-control treatments of the selected plants

The root length enhancement in this research will have physiological basis which may possibly be as a result of the following, according to Howard [12] that the root cortical cells are proliferating at a higher rate; and that an accelerated cell cycle (mitosis) occurred which would have been aided by plant growth hormone such as auxins (it can be said that simulated microgravity enhances and speeds up the work of this growth hormone. Other distinct differences in the growth-rate of the two samples per plant species may also be as a result of the effect of microgravity on plant cellular metabolism [12]. All these involve the flow of information and communications within the underlying cells.

Raghad et al. [5] stated that Clinostat rotation affects plant tissue biochemicals positively which can then be used to enhance plant germination. The gravistimulation produced on corn by Clinostat enhanced the concentration of amino acid than the control [5]. The similar clinorotation experiment carried out by Emmanuel et al. [6] on soybean showed that the root length doubled compared to the stationary control. The result by Jagtap et al. [7] showed that that there was an increase in root length in clinorotated samples and chlorophyll content was also increased in the clinorotated samples as compared to the controls. The study of Lionheart et al. [8] showed that genotype does play a significance in *M. truncatula* morphology affecting plant's response to gravity variation impact.

CONCLUSION

Regarding the evaluated seedlings, cucumber had the highest percentage increase in growth-rate under simulated microgravity by 189.86%, rice had the next highest percentage increase of 104.37% and peanut had 89.60%. These three plants have the most promising results with the use of the microgravity simulations technique. The results from this research also makes an addition to the analytical knowledge of the effect of simulated microgravity on cowpea, peanut, watermelon, okra, cucumber, cotton, wheat, sorghum, corn and rice for future space experiments and missions. From the afore mentioned, it can be affirmed that Clinostat rotation majorly affects plants growth positively by elongating the roots, consequently increasing plant agricultural productivity when such are transferred to the field. As this study dealt only on the morphology (length and curvature) of the root, in furtherance to this research work, biochemical and genetical characterizations will be done on the 1 g and the clinorotated samples. These include (but not limited to) metabolomic proximate analyzes; genes, proteins, enzymes and hormones associated with root germination and elongation; microscopic root anatomy analyzes in order to study the differences in the architectural nature of the root phloem and xylem tissues of the 1 g and the clinorotated-samples. The fresh and dry weight of the roots and also of the aerial part of the plants will also be evaluated in the future to verify the influence of microgravity simulations technique on the accumulation of biomass and not only on the growth of the root. At the long run, it is proposed that the microgravity simulated seedling whose inbuilt architecture of the innate reaction mechanisms have been activated will be transplanted unto the field in order to access their propensity for higher productivity, nutritional qualities and resistance to environmental stresses.

ACKNOWLEDGEMENTS

The United Nations Office for Outer Space Affairs, Vienna, Austria is appreciated for giving a two dimensional Clinostat for research and educational purposes to National Space Research and Development Agency (the Space Agency of Nigeria), Abuja, Nigeria. Authors will also want to sincerely thank Federal University Oye-Ekiti, Nigeria for academic support.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Dunbar B. NASA – Our experiments; 2018. Available:https://www.nasa.gov/spacebio/our-experiments (Accessed 15 July 2020)
2. United Nations. Teacher's guide to plant experiments in microgravity, human space technology initiative. United Nations Programme on Space Applications, New York. Publishing and Library Section, United Nations Office, ST/SPACE/63; 2013.
3. Oluwafemi FA, De La Torre A, Afolayan EM, Olalekan-Ajayi BM, Dhital B, Mora-Almanza JG, et al. Space food and nutrition in a long-term manned mission. Advances in Astronautics Science and Technology; 2018. Available:https://doi.org/10.1007/s42423-018-0016-2
4. RSAM. Root System Anatomy and Morphology; 2017. Available:https://masters.agron.iastate.edu/classes/533/lesson03/3.2.html (Accessed 25 January 2017)
5. Raghad SM, Munawar I, Afzal Q, Lamiaa AM, Ibrahim BR, Mazhar A, Fida H. Effect of gravistimulation on amino acid profile of pea, rice, corn, wheat during early growth stages. Information Processing in Agriculture. 2016;3:244-251.
6. Emmanuel H, Barbara VP, James AG, Christopher SB. Clinorotation affects morphology and ethylene production in soybean. Plant Cell Physiology. 1996;37(7):929-934.
7. Jagtap SS, Awhad RB, Santosh B, et al. Effects of clinorotation on growth and chlorophyll content of rice seeds. Microgravity Sci. Technol. 2011;23:41-48.
8. Lionheart G, Vandenbrink JP, Hoeksema JD, et al. The impact of simulated microgravity on the growth of different genotypes of the model legume plant *Medicago truncatula*. Microgravity Sci. Technol. 2018;30:491-502. Available:https://doi.org/10.1007/s12217-018-9619-4
9. Hoson T, Kamisaka S, Masuda Y, Yamashita M. Changes in plant growth processes under microgravity conditions simulated by a three-dimensional clinostat. The Botanical. 1992;105:53–70.
10. Oluwafemi FA, Olubiyi RA. Investigation of corn seeds growth under simulated microgravity. Arid Zone Journal of Engineering, Technology and Environment (AZOJETE). Centre for Satellite Technology Development Special Issue: Space Science and Technology for Sustainable Development. 2019;15(SP.i2):110-115. Print ISSN: 1596-2490, Electronic ISSN: 2545-5818.
11. UMassAmherst. The Center for Agriculture Food and the Environment Water Analysis.

Available:https://ag.umass.edu/greenhouse-floriculture/greenhouse-best-management-practices-bmp-manual/water-analysis (Accessed 25/08/2020)

12. Howard GL. 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 Pre-Application Meeting, South Shore Harbour Resort & Conference Center, League City, Texas; 2010.