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# Gravity Variation Effects on the Growth of Maize Shoots

**Funmilola Oluwafemi** *

Astrobiology Unit, Space Life Sciences Division, National Space Research and Development Agency (NASRDA), Obasanjo Space Centre, Km 17 Airport Road, P.M.B. 437, Garki, Abuja, Nigeria
* Correspondence: oluwafemifunmilola@gmail.com; Tel.: +23-480-650-35799

**Abstract:** Gravity-variation effects on plants give definite changes. Normal-earth-gravity (1G) and microgravity (μg) are possible variations for experimental purposes. On-board spaceflight microgravity-experiments are rare and expensive, as microgravity-environment is an outstanding platform for research, application and education. Clinostat was used for ground-based experiment to investigate the shoot-morphology of maize plants at the Space-Agency of Nigeria – National Space Research and Development Agency (NASRDA). A Clinostat device uses rotation to negate gravitational-pull effects on plant growth and development. Maize was selected for this experiment because of its nutritional and economic importance; and its usability on the Clinostat. Plant shoot-morphology is important for gravi-responses. Shoot curvature and shoot growth-rate analyzes were done on the shoots of provitamin variety of maize. The seeds were planted into 3 petri-dishes (in-parallels) in a wet-chamber using plant substrate – agar-agar. The experimental conditions were subject to relative-humidity, temperature and light-conditions. After 3 days of germination under 1G, two of the petri-dishes were left under 1G serving as controls for shoot curvature and shoot growth-rate analyzes. The clinorotated-sample was mounted on Clinostat under: fast rotation-speed of 80 rpm, horizontal rotation position and clockwise rotation-direction. The images of the samples were taken 30 minutes interval for 4 hours. After observations, the shoot morphology of the seedlings were studied using ImageJ-software. The grand-average shoot-angles and shoot-lengths of all the seedlings were calculated following the experimental period to give the shoot curvatures and shoot growth-rates respectively. The results showed that the clinorotated-sample had reduced response to gravity with 50.77 /hr for the shoot-curvature while the $90^0$-turned sample had 55.49 /hr. The shoot growth-rate for the 1G-sample was 1.25cm/hr while the clinorotated was 1.26cm/hr. The clinorotated had increased growth-rate per hour than the counterpart 1G. These analytical results serve as a preparation for future real-space experiments on maize and could be beneficial to agriculture-sector.

**Keywords:** Gravity; Microgravity; Clinostat; Maize; Shoot

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## 1. Introduction

Microgravity is known as a condition of assumption of weightlessness. Microgravity research gives insight on the new orientation of plants and materials after been impacted by microgravity. These effects on plants and materials most of the time, gives definite changes on products, which could be beneficial. These researches are therefore called gravity variation researches as the normal-earth-gravity (1G) and microgravity (μg) platforms are possible variations for experimental purposes.

It is apparent that microgravity investigations on plants, cells and organisms can be established beyond doubt only by experiments carried out during space missions, which have limited access and high cost. Similar experiments are now being conducted on the earth surface using microgravity equipment that provides simulated microgravity conditions; such as the Clinostats [1,2]. A Clinostat device uses rotation to negate gravitational-pull effects on plant growth and development. Published reports have shown an increase, a decrease or no significant effect in the growth rate of plants after simulated or real microgravity treatment. Microgravity impact on plants can also have physiological basis [3]. It was stated clearly that under simulated microgravity using Clinostat, the rate of the germination of maize was increased i.e. rotation on the Clinostat did influence the rate of growth of their shoots positively [4,5].

In this work,a 2-D Clinostat (Figure 1) was used as a ground-based research equipment to investigate the shoot morphology of maize (*Zea mays*) seedlings at the Microgravity Simulations Laboratory, National Space Research and Development Agency (NASRDA), Abuja, Nigeria. A two-dimensional (2-D) Clinostat has 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 the Clinostat is called "clinorotation". In this study, the shoot curvature and the growth-rates of maize under simulated microgravity conditions were compared with that under the influence of normal-earth-gravity.


**Figure 1.** 2-D Clinostat at the Microgravity Simulations Laboratory, National Space Research and Development Agency (NASRDA), Abuja, Nigeria.

Maize is number one cereal in Africa and number two cereal in the world [6]; this shows it is an highly important crop. It was also specifically selected for this study due to its small seeds making it easy to handle, and having 3 days germination period. These characteristics makes it useable on the Clinostat. The shoots of the seedlings were also studied because plant shoot morphology is important for gravi-responses. If the shoot of a plant is unable to perform or function, then so will the plant not be able to function.

The aim of this project was to understand the impact of gravity on maize growth to determine what its orientation will be in space, where there is microgravity; as well as to identify the underlying mechanisms and to conduct observational experiments (by measurement of the curvature angles and growth-rates of shoots using ImageJ software) with respect to gravitropic reactions with the shoots grown of maize under simulated microgravity environment and comparing them with those of control experiments.

## 2. Experiments

Maize seeds were bought and authenticated to be the actual seeds sought after. The substrate of the seeds called plant agar-agar was prepared into 3 petri-dishes using the standard preparation method in the Teacher's Guide to Plant Experiments by UNOOSA of the Programme on Space Applications) [7]; then the maize seeds were planted in the substrate (9 seeds per petri-dish; 3 seeds in parallels) by been held in alignment with the direction of the gravity vector, in a wet chamber for cultivation (Figure 2). After 3 days, germination of the seeds with short shoots were observed. The 3 petri-dishes were then taken and labeled “1G-control”, “$90^0$-turned” and “clinorotated”. The 1G-control sample was remained in the alignment with the direction of gravity vector. The $90^0$-turned sample was rotated by $90^0$ and the clinorotated sample was then placed at the centre of the Clinostat using double-sided tape. The 1G-control sample served as the control for the clinorotated sample for the growth-rate analysis while the $90^0$-turned sample served as the control for the clinorotated sample to authenticate if gravity is really active in the laboratory room.

**Figure 2.** The maize seeds in the wet chamber for cultivation.

The photos of the 3 petri-dishes were taken every 30 minutes (during the period of observation) (Figures 3). These observations were done for 4hr under the following conditions. Humidity of 75%, temperature of $23^0$C and light of 60lx. In addition to these, the clinorotated sample had the following conditions: rotation-speed of 80rpm (fast rotation speed), horizontal rotation position and the direction of rotation was clockwise. At the end of observation, the analysis of shoot curvature and growth-rate were carried out.

(a) (b) (c)

**Figure 3.** The three samples: (**a**) 1G-control sample; (**b**) $90^0$-turned sample; (c) Clinorotated sample.

## 3. Results

The data obtained were the three sets of images of the shoots which show the “1G-control”, “90°-turned” and “clinorotated” shoots (Figure 3). An image-processing application software called ImageJ was used to analyse the set of images.

### *3.1. Shoot growth-rate*

The pictures of the 1G-control and the clinorotated shoots were used for the shoot growth-rate analysis. The difference between the two cases was analyzed by measuring the length of the shoots, which thereby allowed their growth-rate to be determined. The length of the shoots were gotten by using the length measurement tool of the ImageJ software. The average growth-rate were then calculated (centimetres/hour) for the 1G-control and the clinorotated shoots.

The average growth-rate of the shoots for the 1G-control sample was 1.25cm/hr while that of the clinorotated sample was 1.26cm/hr. Table 1 shows the shoot lengths and Figure 4 shows the graph of the averaged shoot lengths of the 1G-control and the clinorotated shoots samples.

**Table 1.** Seedling shoot length of the 1G-control and the clinorotated samples.

| **1G-control** | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | **0 hr** | **0.5 hr** | **1 hr** | **1.5 hr** | **2 hr** | **2.5 hr** | **3 hr** | **3.5 hr** | **4 hr** |
| Seed1 (cm) | | | | | | | | | |
| Seed2 (cm) | 1.11 | 1.093 | 1.202 | 1.144 | 1.493 | 1.397 | 1.256 | 1.36 | 1.692 |
| Seed3 (cm) | 0.673 | 0.997 | 1.039 | 0.897 | 1.03 | 0.994 | 0.885 | 1.031 | 1.152 |
| Seed4 (cm) | | | | | | | | | |
| Seed5 (cm) | 1.623 | 1.439 | 1.762 | 1.397 | 1.888 | 1.778 | 1.675 | 1.719 | 2.037 |
| Seed6 (cm) | 0.669 | 0.69 | 0.981 | 0.722 | 0.951 | 0.864 | 0.718 | 1.029 | 0.883 |
| Seed7 (cm) | | | | | | | | | |
| Seed8 (cm) | 1.328 | 1.269 | 1.555 | 1.201 | 1.775 | 1.396 | 1.34 | 1.499 | 1.783 |
| Seed9 (cm) | | | | | | | | | |
| **Average (cm)** | **1.0806** | **1.0976** | **1.3078** | **1.0722** | **1.4274** | **1.2858** | **1.1748** | **1.3276** | **1.5094** |
| **Clinorotated** | | | | | | | | | |
| | **0 hr** | **0.5 hr** | **1 hr** | **1.5 hr** | **2 hr** | **2.5 hr** | **3 hr** | **3.5 hr** | **4 hr** |
| Seed1 (cm) | 1.612 | 1.796 | 1.82 | 1.929 | 1.754 | 1.806 | 2.33 | 2.178 | 2.468 |
| Seed2 (cm) | | | | | | | | | |
| Seed3 (cm) | 0.652 | 0.927 | 0.788 | 0.877 | 0.923 | 1.166 | 1.191 | 1.412 | 1.335 |
| Seed4 (cm) | | | | | | | | | |
| Seed5 (cm) | | | 0.858 | 0.9 | 0.98 | 0.998 | 1.191 | 1.239 | 1.419 |
| Seed6 (cm) | 0.6 | 0.778 | 0.827 | 0.741 | 0.748 | 0.769 | 1.145 | 0.935 | 0.9 |
| Seed7 (cm) | 1.337 | 1.486 | 1.555 | 1.584 | 1.493 | 1.45 | 1.789 | 1.619 | 1.986 |
| Seed8 (cm) | | | | | | | | | |
| Seed9 (cm) | 0.901 | 0.909 | 0.819 | 0.952 | 0.996 | 1.153 | 1.226 | 1.263 | 1.533 |
| **Average (cm)** | **1.0204** | **1.1792** | **1.111167** | **1.163833** | **1.149** | **1.223667** | **1.478667** | **1.441** | **1.606833333** |

*Some of the seeds didn't grow, therefore their spaces remained empty.

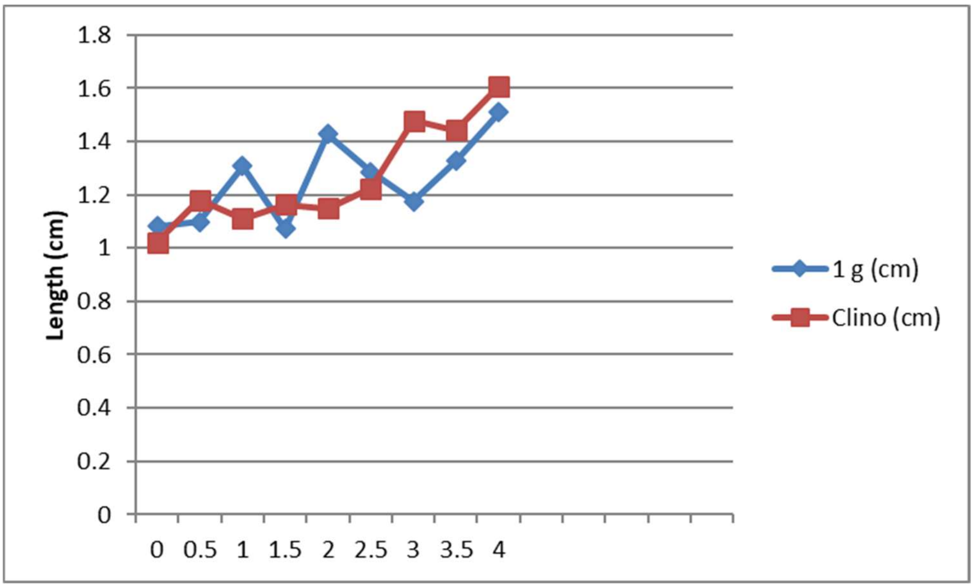


**Figure 4.** Shoot length of the 1G-control and the clinorotated samples of maize seedlings.

### *3.2. Shoot curvature*

The shoot curvature analysis focuses on the curvature of the shoots which are photos of the $90^0$-turned and the clinorotated samples. All the curvature angles of the shoots were measured using the angle measurement tool in the software. The average angular rate of the shoot bending in degrees per hour was then calculated.

The images of the 90°-turned sample showed that the shoots started bending in the direction of gravity after the petri-dish was turned by $90^0$. The clinorotated shoot did not show much bending as much as the $90^0$-turned. The average angular rate of the shoot bending for the $90^0$-turned was $55.49^0$/hr while that of the clinorotated was $50.77^0$/hr. Table 2 shows the degrees of curvature of the shoots and Figure 5 shows the bar chart in degrees of the averaged curvature of the shoots of the $90^0$-turned and the clinorotated samples.

**Table 2.** Seedling shoot curvature of the $90^0$-turned and the clinorotated samples.

| **90°-turned** | **0 hr** | **0.5 hr** | **1 hr** | **1.5 hr** | **2 hr** | **2.5 hr** | **3 hr** | **3.5 hr** | **4 hr** |
|---|---|---|---|---|---|---|---|---|---|
| Seed1 (°) | | | | | | | | | |
| Seed2 (°) | 121.144 | 115.56 | 125.224 | 139.841 | 100.631 | 112.937 | 114.204 | 100.305 | 100.924 |
| Seed3 (°) | | | | | | | | | |
| Seed4 (°) | | | | | | | | | |
| Seed5 (°) | | | | | | | | | |
| Seed6 (°) | 164.208 | 135 | 136.062 | 119.959 | 112.834 | 114.655 | 140.293 | 103.861 | 103.054 |
| Seed7 (°) | 137.629 | 155.65 | 126.806 | 104.036 | 65.659 | 78.69 | 120.411 | 77.558 | 103.274 |
| Seed8 (°) | 136.204 | 137.873 | 131.675 | 125.948 | 100.106 | 91.259 | 88.838 | 88.831 | 76.058 |
| Seed9 (°) | 151.526 | 158.499 | 166.102 | 165.964 | 173.29 | 162.049 | 173.411 | 176.634 | 168.149 |
| **Average (°)** | **142.1422** | **140.5164** | **137.1738** | **131.1496** | **110.504** | **111.918** | **127.4314** | **109.4378** | **110.2918** |
| **Real Curvature Angle (180-Average) (cm)** | **37.8578** | **39.4836** | **42.8262** | **48.8504** | **69.496** | **68.082** | **52.5686** | **70.5622** | **69.7082** |
| **Clinorotated** | | | | | | | | | |
| | **0 hr** | **0.5 hr** | **1 hr** | **1.5 hr** | **2 hr** | **2.5 hr** | **3 hr** | **3.5 hr** | **4 hr** |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Seed1 (°) | 110.453 | 119.876 | 102.995 | 90.41 | 91.573 | 90 | 80.011 | 75.161 | 59.59 |
| Seed2 (°) | | | | | | | | | |
| Seed3 (°) | 151.849 | 130.752 | 88.315 | 92.837 | 94.557 | 78.234 | 110.032 | 97.053 | 76.009 |
| Seed4 (°) | | | | | | | | | |
| Seed5 (°) | | | 166.027 | 171.508 | 153.965 | 139.344 | 132.897 | 154.964 | 149.371 |
| Seed6 (°) | 123.896 | 124.509 | 140.194 | 152.411 | 149.092 | 136.613 | 107.038 | 126.893 | 142.888 |
| Seed7 (°) | 133.452 | 136.325 | 153.158 | 157.769 | 145.988 | 150.652 | 166.017 | 163.562 | 145.78 |
| Seed8 (°) | | | | | | | | | |
| Seed9 (°) | 176.566 | 123.111 | 130.365 | 171.741 | 167.922 | 173.498 | 153.69 | 156.252 | 94.83 |
| **Average** | **139.2432** | **126.9146** | **130.1757** | **139.446** | **133.8495** | **128.0568** | **124.9475** | **128.9808** | **111.4113** |
| **Real Curvature Angle (180-Average) (cm)** | **40.7568** | **53.0854** | **49.82433** | **40.554** | **46.1505** | **51.94317** | **55.0525** | **51.01917** | **68.58867** |

*Some of the seeds didn't grow, therefore their value spaces remained empty.

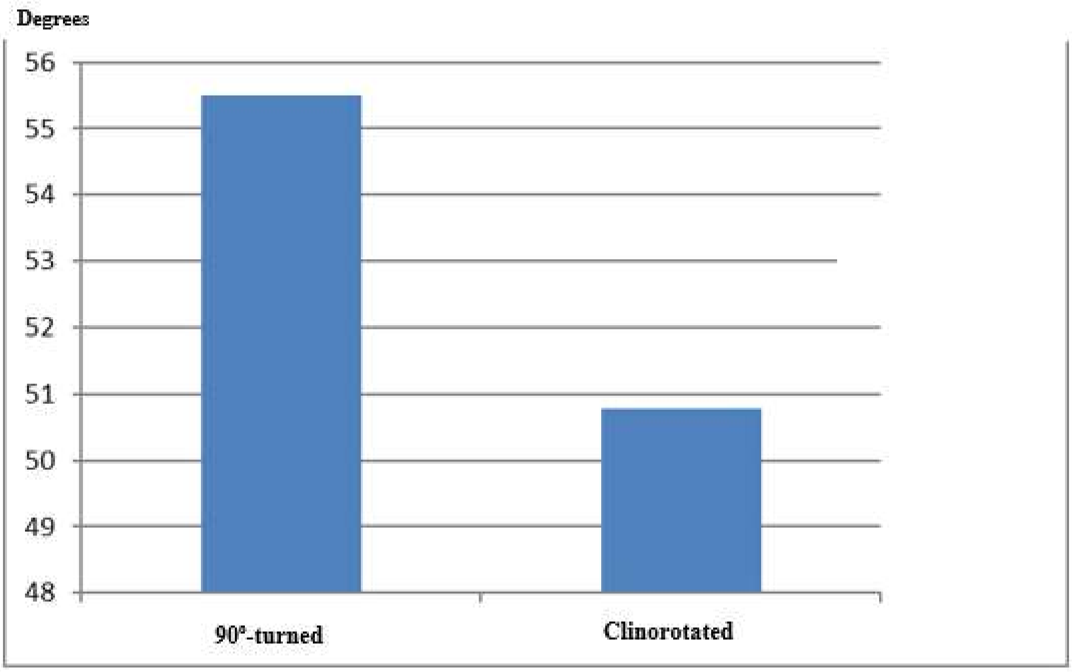


**Figure 5.** Shoot curvature of the $90^0$-turned and the clinorotated samples of maize seedlings.

## 4. Discussion

The images of the 1G-control showed that the shoots continuously grew in the direction of gravity. The shoots of the 1G-control and the clinorotated appeared to be similar, but the mechanisms for stimulating their growth are totally different in each case. For the 1G-control, the earth's gravity continuously stimulated the growth of the shoots in the direction of gravity. For the clinorotated shoots, however, nothing stimulated their growth in any direction.

The shoot length enhancement have physiological basis which may possibly be as a result of the following (Howard, 2010): the shoot cortical cells proliferating at a higher rate; an accelerated cell cycle (mitosis) which would have been aided by plant growth hormones such as auxins (it can be said that simulated microgravity enhances and speeds up the work of growth hormones); and that microgravity environment disrupts normal carbohydrate metabolism affecting the shoot cell structure. It can be deduced that there could be changes in the vascular structure of the shoots as a result of the orientation of microfibrils and their assembly in developing vessels perturbed by simulated microgravity.

The image of the 90°-turned sample showed that the shoots started bending in the direction of gravity after the petri-dish was turned by 90°. This is an evidence of gravitropism of the shoots; this indicates a positive response to simulated microgravity. This was evident from the growing direction of the shoots which was random under simulated microgravity while the shoot tips of the 90°-turned bent vertically upwards.

Therefore, maize has a promising result with the use of Clinostat simulated microgravity model. This study is only on the shoot morphology (curvature and length); further research work is proposed on the plant photosynthesis, respiration, transpiration, and gene expression. All these involve the flow of information and communications within the underlying cells.

## 5. Conclusions

Plants account for the majority of human food. Therefore, improving the growth-rate status of plants will help increase the crop's yield which is an important factor to feeding the world's growing population. In this study, simulated microgravity using 2-D Clinostat was able to cause an increase in the shoot growth-rate of maize as a response from gravity to simulated microgravity. Therefore, simulated microgravity of Clinostat is proposed to have beneficial effects on the in-built structure of seedlings before they are transplanted unto the field to produce better product yields and higher nutritional qualities. Thus, "simulated space stressing"of plant at the early stage of seedling could be advantageous.

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**Conflicts of Interest:** The author declares no conflict of interest.

## Abbreviations

The following abbreviations are used in this manuscript:

1G: Normal-Earth-Gravity
μg: Microgravity
NASRDA: National Space Research and Development Agency (NASRDA)
UNOOSA: United Nations Office for Outer Space Affairs
2-D: Two-Dimensional

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