Gravity Variation Effects on the Growth of Maize Shoots †

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Abstract: Gravity variation effects on plants provide definite changes. Normal Earth gravity (1G) and microgravity (µg) are possible variations for experimental purposes. On-board spacelift microgravity experiments are rare and expensive, as the microgravity environment is an outstanding platform for research, application and education. A Clinostat was used for ground-based experiments 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 analyses were conducted on the shoots of a provitamin variety of maize. The seeds were planted into three Petri dishes (in parallel) in a wet chamber using a 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 analyses. The clinorotated sample was mounted on the Clinostat under: a fast rotation speed of 80 rpm, a horizontal rotation position and a clockwise rotation direction. The images of the samples were taken at a 30 min interval for 4 h. After observations, the shoot morphology of the seedlings was studied using ImageJ software. The grand average shoot angles and shoot lengths of all the seedlings were calculated following the experimental period to provide the shoot curvatures and shoot growth rates, respectively. The results show that the clinorotated sample had a reduced response to gravity, with 50.77°/h for the shoot curvature, while the 90°-turned sample had 55.49°/h. The shoot growth rate for the 1G sample was 1.25 cm/h, while that for the clinorotated sample was 1.26 cm/h. The clinorotated sample had an increased growth rate per hour compared to the counterpart 1G sample. These analytical results serve as preparation for future real-space experiments on maize and could be beneficial to the agriculture sector.

Keywords: gravity; microgravity; Clinostat; maize; shoot

1. Introduction

Microgravity is known as a condition of the assumption of weightlessness. Microgravity research provides insight on the new orientation of plants and materials after being impacted by microgravity. These effects on plants and materials, most of the time, cause definite changes in products, which could be beneficial. This research is therefore called gravity variation research, 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 a high cost. Similar experiments are now being conducted on the Earth’s 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. The microgravity impact on plants can also have a physiological basis [3]. It was stated clearly that under simulated microgravity using a Clinostat, the rate of the germination of maize was increased, i.e., rotation on the Clinostat did influence the rate of growth of maize shoots positively [4,5].

In this work, a 2-D Clinostat (Figure 1) was used as a ground-based research instrument 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 the speed and direction of the rotation. A rotation on the Clinostat is called a “clinorotation”. In this study, the shoot curvature and the growth rates of maize under simulated microgravity conditions were compared with those under the influence of normal Earth gravity.

Maize is the number one cereal in Africa and number two cereal in the world [6]; this shows it is a highly important crop. It was also specifically selected for this study due to its small seeds, making it easy to handle, and having a 3-day 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 graviresponses. If the shoot of a plant is unable to perform or function, then the plant will not be able to function either.

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, 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 maize shoots grown under a simulated microgravity environment, comparing them with those of the 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 placed 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 parallel) by being 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 was observed. The 3 Petri dishes were then taken and labeled “1G-control”, “90°-turned” and “clinorotated”. The 1G-control sample remained in alignment with the direction of the gravity vector. The 90°-turned sample was rotated by 90°, and the clinorotated sample was then placed at the center 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°-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.](image)

At the end of observation, the analyses of shoot curvature and growth rate were carried out. The photos of the 3 Petri dishes were taken every 30 min (during the period of observation) (Figure 3). These observations were carried out for 4 h under the following conditions. Humidity of 75%, temperature of 23 °C and light of 60 lx. In addition to these, the clinorotated sample had the following conditions: rotation speed of 80 rpm (fast rotation speed), a horizontal rotation position and the direction of rotation was clockwise. At the end of observation, the analyses of shoot curvature and growth rate were carried out.

![Figure 3. The three samples: (a) 1G-control sample; (b) 90°-turned sample; (c) clinorotated sample.](image)

### 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 analyze 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 was obtained by using the length measurement tool of the ImageJ software. The average growth rate was then calculated (centimeters/hour) for the 1G-control and the clinorotated shoots.

The average growth rate of the shoots for the 1G-control sample was 1.25 cm/h, while that of the clinorotated sample was 1.26 cm/h. 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.

| Seed   | 0 h  | 0.5 h | 1 h  | 1.5 h | 2 h  | 2.5 h | 3 h  | 3.5 h | 4 h  |
|--------|------|-------|------|-------|------|-------|------|-------|------|
| 1G-Control |      |       |      |       |      |       |      |       |      |
| Seed1  | 1.11 | 1.093 | 1.202| 1.144 | 1.493| 1.397 | 1.256| 1.36  | 1.692|
| Seed2  | 0.673| 0.997 | 1.039| 0.897 | 1.03 | 0.994 | 0.885| 1.031 | 1.152|
| Seed3  | 1.623| 1.439 | 1.762| 1.397 | 1.888| 1.778 | 1.675| 1.719 | 2.037|
| Seed4  | 0.669| 0.69  | 0.981| 0.722 | 0.951| 0.864 | 0.718| 1.029 | 0.883|
| Seed5  | 1.328| 1.269 | 1.555| 1.201 | 1.775| 1.396 | 1.34 | 1.499 | 1.783|
| Seed6  | 1.623| 1.439 | 1.762| 1.397 | 1.888| 1.778 | 1.675| 1.719 | 2.037|
| Seed7  | 0.669| 0.69  | 0.981| 0.722 | 0.951| 0.864 | 0.718| 1.029 | 0.883|
| Seed8  | 1.328| 1.269 | 1.555| 1.201 | 1.775| 1.396 | 1.34 | 1.499 | 1.783|
| Seed9  | 1.623| 1.439 | 1.762| 1.397 | 1.888| 1.778 | 1.675| 1.719 | 2.037|
| Average| 1.0806| 1.0976| 1.3078| 1.0722| 1.4274| 1.2858| 1.1748| 1.3276| 1.5094|

| Clinorotated | 0 h  | 0.5 h | 1 h  | 1.5 h | 2 h  | 2.5 h | 3 h  | 3.5 h | 4 h  |
|--------------|------|-------|------|-------|------|-------|------|-------|------|
| Seed1        | 1.612| 1.796 | 1.82 | 1.929 | 1.754| 1.806 | 2.33 | 2.178 | 2.468|
| Seed2        | 0.652| 0.927 | 0.788| 0.877 | 0.923| 1.166 | 1.191| 1.412 | 1.335|
| Seed3        | 0.858| 0.9  | 0.98 | 0.98  | 0.998| 1.191 | 1.239| 1.419 | 1.419|
| Seed4        | 0.6  | 0.778 | 0.827| 0.741 | 0.748| 0.769 | 1.145| 0.935 | 0.9  |
| Seed5        | 1.337| 1.486 | 1.555| 1.584 | 1.493| 1.45  | 1.789| 1.619 | 1.986|
| Seed6        | 0.901| 0.909 | 0.819| 0.952 | 0.996| 1.153 | 1.226| 1.263 | 1.533|
| Seed7        | 1.0204| 1.1792| 1.111167| 1.163833| 1.149| 1.223667| 1.478667| 1.441| 1.606833333|
| Average      | 1.0806| 1.0976| 1.3078| 1.0722| 1.4274| 1.2858| 1.1748| 1.3276| 1.5094|

Some of the seeds did not grow; therefore, their spaces remain empty.

Figure 4. Shoot length (cm) of the 1G-control and the clinorotated samples of maize seedlings against the time (h).

3.2. Shoot Curvature

The shoot curvature analysis focuses on the curvature of the shoots through photos of the 90°-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 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°. The clinorotated shoot did not show much bending compared to the 90°-turned sample. The average angular rate of shoot bending for the 90°-turned sample was 55.49°/h, while that of the clinorotated sample was 50.77°/h. 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°-turned and the clinorotated samples.

Table 2. Seedling shoot curvature of the 90°-turned and the clinorotated samples.

| 90°-Turned | 0 h  | 0.5 h | 1 h  | 1.5 h | 2 h  | 2.5 h | 3 h  | 3.5 h | 4 h  |
|------------|------|-------|------|-------|------|-------|------|-------|------|
| Seed1 (°)  | 121.144 | 115.56 | 125.224 | 139.841 | 100.631 | 112.937 | 114.204 | 100.305 | 100.924 |
| Seed2 (°)  | 164.208 | 135 | 136.062 | 119.959 | 112.834 | 114.655 | 140.293 | 103.861 | 103.054 |
| Seed3 (°)  | 137.629 | 155.65 | 126.806 | 104.036 | 65.659 | 78.69 | 120.411 | 77.558 | 103.274 |
| Seed4 (°)  | 136.204 | 137.873 | 131.675 | 125.948 | 100.106 | 91.259 | 88.838 | 88.831 | 76.038 |
| Seed5 (°)  | 151.526 | 158.499 | 166.102 | 165.964 | 173.29 | 162.049 | 173.411 | 176.634 | 168.149 |
| Seed6 (°)  | 133.452 | 136.325 | 153.158 | 157.769 | 145.988 | 152.411 | 149.371 | 107.038 | 142.888 |
| Seed7 (°)  | 123.896 | 124.509 | 140.194 | 152.411 | 149.092 | 136.613 | 154.964 | 126.893 | 145.78 |
| Seed8 (°)  | 176.566 | 123.111 | 130.365 | 171.741 | 167.922 | 173.498 | 153.69 | 156.252 | 94.83 |
| Seed9 (°)  | 139.2432 | 126.9146 | 130.1757 | 139.446 | 133.8495 | 128.0568 | 124.9475 | 128.9808 | 111.4113 |
| Average (°) | 142.1422 | 140.5164 | 137.1738 | 131.1496 | 110.504 | 111.918 | 127.4314 | 109.4378 | 110.2918 |
| Real Curvature Angle (180-Average) (°) | 37.8578 | 39.4836 | 42.8262 | 48.8504 | 69.496 | 68.082 | 52.5686 | 70.5622 | 69.7082 |

| Clinorotated | 0 h  | 0.5 h | 1 h  | 1.5 h | 2 h  | 2.5 h | 3 h  | 3.5 h | 4 h  |
|--------------|------|-------|------|-------|------|-------|------|-------|------|
| Seed1 (°)    | 110.453 | 119.876 | 102.995 | 90.41 | 91.573 | 90 | 80.011 | 75.161 | 59.59 |
| Seed2 (°)    | 151.849 | 130.752 | 88.315 | 92.837 | 94.557 | 78.234 | 110.032 | 97.053 | 76.009 |
| Seed3 (°)    | 166.027 | 171.508 | 153.965 | 139.344 | 132.897 | 154.964 | 149.371 | 126.893 | 142.888 |
| Seed4 (°)    | 123.896 | 124.509 | 140.194 | 152.411 | 149.092 | 136.613 | 154.964 | 126.893 | 145.78 |
| Seed5 (°)    | 133.452 | 136.325 | 153.158 | 157.769 | 145.988 | 152.411 | 149.092 | 136.613 | 154.964 |
| Seed6 (°)    | 176.566 | 123.111 | 130.365 | 171.741 | 167.922 | 173.498 | 153.69 | 156.252 | 94.83 |
| Seed7 (°)    | 139.2432 | 126.9146 | 130.1757 | 139.446 | 133.8495 | 128.0568 | 124.9475 | 128.9808 | 111.4113 |
| Average (°)  | 139.2432 | 126.9146 | 130.1757 | 139.446 | 133.8495 | 128.0568 | 124.9475 | 128.9808 | 111.4113 |
| Real Curvature Angle (180-Average) (°) | 40.7568 | 53.0854 | 49.82433 | 40.554 | 46.1505 | 51.94317 | 55.0525 | 51.01917 | 68.58867 |

Some of the seeds did not grow; therefore, their value spaces remain empty.

Figure 5. Shoot curvature of the 90°-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 samples 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 has a physiological basis which may possibly be 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 the 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 evidence of gravitropism of the shoots; this indicates a positive response to the 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 sample bent vertically upwards.

Therefore, maize has promising results with the use of a Clinostat simulated microgravity model. This study was only focused on the shoot morphology (curvature and length); further research work is proposed on 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 crops’ yields, which is an important factor to feeding the world’s growing population. In this study, simulated microgravity using a 2-D Clinostat was able to cause an increase in the shoot growth rate of maize as a response of gravity to the simulated microgravity. Therefore, the simulated microgravity of the 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 plants at the early stage of seedlings could be advantageous.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/21/s1.

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

Abbreviations

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

References

1. Afolayan, E.M.; Oluwafemi, F.A.; Jeff-Agboola, E.O.; Oluwasegun, T.; Ayankale, J.O. Socio-economic benefits of microgravity research. Centre for Satellite Technology Development Special Issue: Space Science and Technology for Sustainable Development. Arid Zone J. Eng. Technol. Environ. AZOJETE 2019, 15, 57–74, Print ISSN 1596-2490, Electron vic ISSN 2545-5818.

2. Oluwafemi, F.A.; De La Torre, A.; Afolayan, E.M.; Olalekan-Ajayi, B.M.; Dhital, B.; Mora-Almanza, J.G.; Potrivitu, G.; Creech, J.; Rivolta, A. Space food and nutrition in a long-term manned mission. Adv. Astronaut. Sci. Technol. 2018, 1, 1. [CrossRef]
3. Howard, G.L. The Influence of microgravity on plants. NASA Surface Systems Office. In 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, TX, USA, 2010.

4. Oluwafemi, F.A.; Ibraheem, O.; Fatoki, T.H. Clinostat microgravity impact on shoot morphology of selected nutritional and economic crops. Plant Cell Biotechnol. Mol. Biol. 2020, 21, 92–104.

5. Oluwafemi, F.A.; Olubiyi, R.A. Investigation of corn seeds growth under simulated microgravity. Centre for Satellite Technology Development Special Issue: Space Science and Technology for Sustainable Development. Arid Zone J. Eng. Technol. Environ. AZOJETE 2019, 15, 110–115, Print ISSN 1596-2490, Electronic ISSN 2545-5818.

6. Awika, M.J. Major cereal grains production and use around the world. ACS Symp. Ser. 2011, 1089, 1–13. [CrossRef]

7. United Nations. Teacher’s Guide to Plant Experiments in Microgravity. Human Space Technology Initiative; ST/SPACE/63; United Nations Programme on Space Applications, Publishing and Library Section, United Nations Office: New York, NY, USA, 2013.