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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 spaceflight 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

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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.

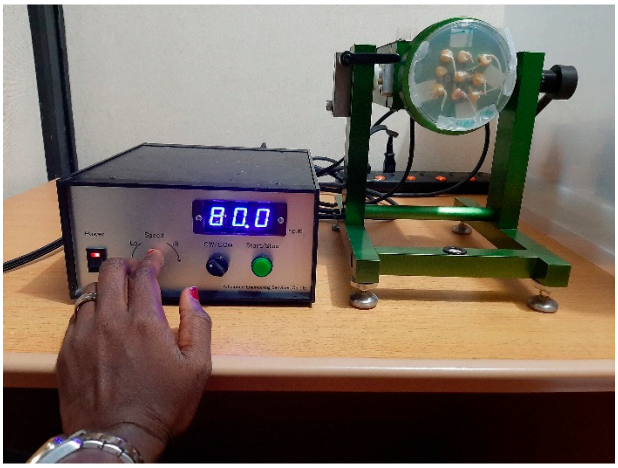

Figure 1. 2-D Clinostat at the Microgravity Simulations Laboratory, National Space Research and Development Agency (NASRDA), Abuja, Nigeria.

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 gravi-responses. 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.

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.

(**a**) (**b**) (**c**)

Figure 3. The three samples: (**a**) 1G-control sample; (**b**) 90°-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 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.

1G-Control									
	0 h	**0.5 h**	**1 h**	**1.5 h**	**2 h**	**2.5 h**	**3 h**	**3.5 h**	**4 h**
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 h	**0.5 h**	**1 h**	**1.5 h**	**2 h**	**2.5 h**	**3 h**	**3.5 h**	**4 h**
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 did not grow; therefore, their spaces remain empty.

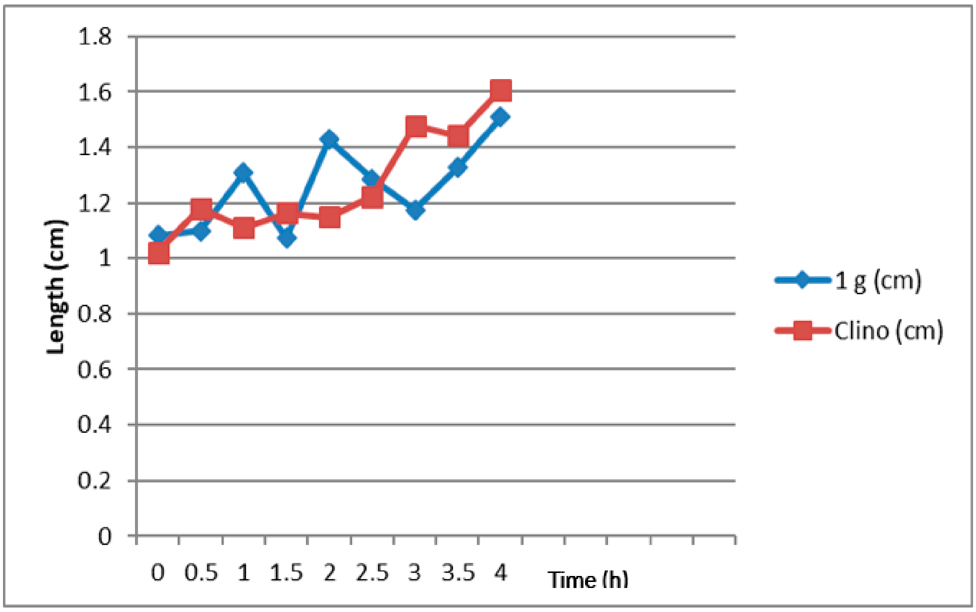

Figure 4. Shoot length (cm) of the 1G-control and the clinorotated samples of maize seedlings against the time (h).

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 (°)									
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) (°)	**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 (°)									
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) (°)	**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.

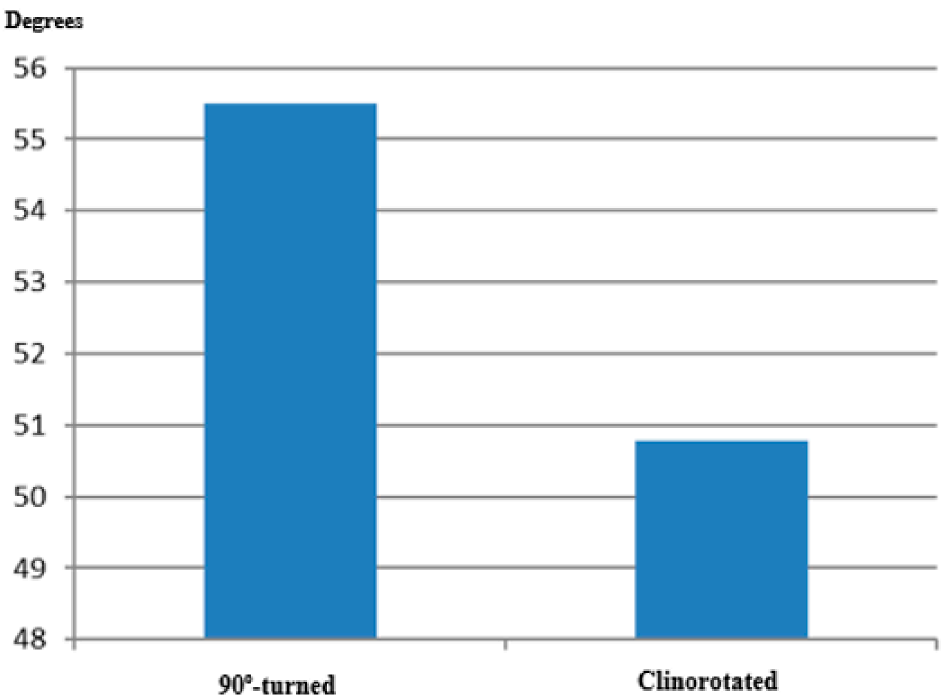

Figure 5. Shoot curvature of the 90°-turned and the clinorotated samples of maize seedlings.

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

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