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Research Article

Microgravity-Induced Morphological Differences in Cowpea Seedlings

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Abstract

Background: Understanding the pattern of plant growth in microgravity environment is important for space science and sustainability of life in space environment. The aim of this study is to determine the effect of clinorotation on cowpea seedlings. **Method:** Cowpea seedlings were soaked in 3% bleach solution for 10 minutes and rinsed off 3-5 times with running water. Agar solution was prepared and poured in three petri dishes. The seeds were randomly assigned to the three petri dishes representing horizontal, 90° and clinorotation placement. Plants were subjected to treatment and observed for 24 and 48 hours. Data was collected and analyzed using SPSS. **Result:** After 24 hours, short root growth was observed in all groups. After 48 hours, root growth was higher in clinorotated and 90° placed compared to horizontally placed seedlings. The median root growth was higher in 90° (4.45cm) and clinorotated (2.78cm) compared to horizontally (2.59cm) placed groups. Also, median root curvature was higher in clinorotated (65.02°) and 90° (60.94°) compared horizontally (57.57°) placed group. The median root growth and curvature after 24 and 48 hours were not statistically significantly different ($P>.05$). Although the difference in the median root curvature was not statistically different after 24 hours ($P>.05$), this was statistically significantly different between groups after 48 hours ($P<.05$). **Conclusion:** This study shows that while there was no significant difference in root growth, clinorotation had significant effect on the curvature of cowpea seedlings. It is important to study the long-term effect of microgravity on other morphologic, biochemistry, proximate and nutritional parameters.

1. Introduction

For decades, scientists across the globe have explored how life can function and adapt in space environment. This is because the space environment is characterized by extreme conditions such as microgravity, radiation vacuum and fluctuation in temperature. Microgravity is characterized by the presence of very low gravitational force that alters the dynamics of fluid, thermal convection and diffusion of gases in living organisms [1, 2]. Because biological processes such as nutrient transport, uptake of water and morphology of plants depend on

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the earth's gravity, plants may rely on conditions such as light, hormonal signaling and moisture to adapt to stresses due to this altered environmental condition [3]. Thus, understanding how plants adapt to these conditions could promote sustainability of life and long-term space mission.

The possibility of plant development in a microgravity environment has been the subject of numerous research initiatives. *Arabidopsis thaliana (L)* seedlings germinated and finished their life cycle regardless of the microgravity situation, according to a seed-to-seed study carried out in a cell biology experiment facility on a Japan module of the International Space Station [4]. Another study conducted on Spacelab II found that the rate of formation of lignin in mung beans and oat seedlings reduced with microgravity which resulted in lower growth rate and stem and root disruption [5]. Other studies have found alteration in the genetics, morphology and cellular differences of plants subjected under microgravity [6–9]. These studies have provided evidence on the resilience of plants to microgravity and a basis for emerging exploration in space agriculture especially amongst crops that are largely produced and are negatively affected by environmental changes.

Cowpea *(Vigna unguiculata)* is one of the plant seedlings that is best suited for microgravity study because of its short germination period, quick seed growth, and significance as a widely consumed staple. This plant is one of the most ancient important food source and forage legume crops in the world [10–12]. It is a self-pollinating dicotyledonous crop plant that has a unique gravitotropic feature that are predictable which is seen in its downward primary root growth and upward shoot growth under gravity condition [13]. Planting cowpea under simulated microgravity environment may potentially display morphological differences as identified in previous studies. One study conducted to assess the effect of clinorotation on the morphological differences of selected Nigerian crops found that cowpea subjected to microgravity simulation on a clinostat for 6½ hours had higher root growth-rate and a lesser curvature per hour compared to plants that were subjected to 90° positioning, identifying the potential of cowpea thriving morphologically under microgravity environment [14]. Unlike previous research where seedlings were pre-grown before mounting on the clinostat, it is important that studies are conducted to observe the effect of immediate clinorotation, in order to understand the developmental variations with earliest microgravity induction while eliminating the prior root orientation before clinorotation.

Thus, the purpose of this study is to evaluate how cowpea seedling morphology is affected by simulated microgravity (using a clinostat). The study's specific goals will be as follows:

- To determine the effect of clinorotation on short root growth of cowpea seedlings.
- To assess the effect of clinorotation root curvature of cowpea seedlings.
- To compare the differences in root growth and curvature of cowpea seedlings after simulation of microgravity with horizontal placement and 90° positioning.

2. Materials ansd Methods

2.1. Experimental Design

The seedlings of cowpea (SAMPEA 14) was purchased from the International Institute of Tropical Agriculture, Federal Capital Territory (FCT), Nigeria. The experiment was conducted in the NASRDA Abuja life sciences laboratory from November 7–9, 2025.

2.2. Materials and Equipment

The materials used for this project include cowpea (the same cultivar, age and size), petri dishes, nutrient medium (agar), 3% sodium hypochlorite, forceps, glass rod, wetting agent, paper towels, transparent stick film, permanent markers, heating plate, pH strip, thermometer, wet chamber, weighing scale, hygrometer, camera, 2D clinostat, wooden holders, measuring tools (ruler, caliper, digital camera, analytic balance for fresh and dry mass), data collection device (laptop), data management and analytic software (Microsoft Excel, SPSS).

2.3. Study Procedure and Assignment

Bleaching

About 3% of bleach solution was prepared to sterilized seedlings as demonstrated below;

3% Sodium hypochlorite = 3ml Sodium hypochlorite + 97ml distilled water

The seeds (cowpea) were soaked in this solution for 10 minutes and rinsed off 3 times with running tap water. The seeds were randomly assigned to groups (G1, G2 and G3).

Preparation of Growth Medium

The medium was prepared by adding 3g of agar-agar powder into a 2 liter of tap water in a beaker [14]. The mixture was stirred using a glass rod until well combined. The beaker was heated on a hot plate under low heat with continuous stirring until agar is completely dissolved and the solution was clear [15]. The pH of the solution was measured using a pH strip. A gradual addition of hydrochloric acid (HCL) was used to adjust the pH from 6 to give a measurement of 5. The solution was let to rest for five minutes add was transferred into each sterile petri dish (G1, G2, G3) to sit at room temperature [15]. These mediums were labelled G1, G2, and G3 (Table 1).

Table 1: Assignment of Seedlings into Experimental Groups

Group	Treatment
G1	Control (horizontal)
G2	Control (vertically inverted at 90°)
G3	Clinorotated

Note: G=Group

A total of 9 seeds were placed in each petri dish and all seeds were placed in the same direction. Prior to the placement of seeds, all petri dishes were placed on a template and a vertical reference line was drawn indicating the direction of gravity as explained in the teacher manual [15]. The petri dish was covered with a lid and transparent stick films were used to seal the petri dishes with gaps for oxygen support. After preparation, all petri dishes were immediately assigned to their experimental groups to observe the direct effect of experimentation on germination and seed morphology. As a result, G1 was placed vertically on a wooden holder at 90°, and G2 horizontally in a wet chamber (plastic box of 40cm x 40cm x 40cm dimension). The humidity of the chamber was maintained as specified by the teachers guideline (75-78%) [15]. These samples were monitored for 24-48 hours for visible short root growth (5mm-10mm) and the study outcomes (morphological differences) [15] (Figure 1).

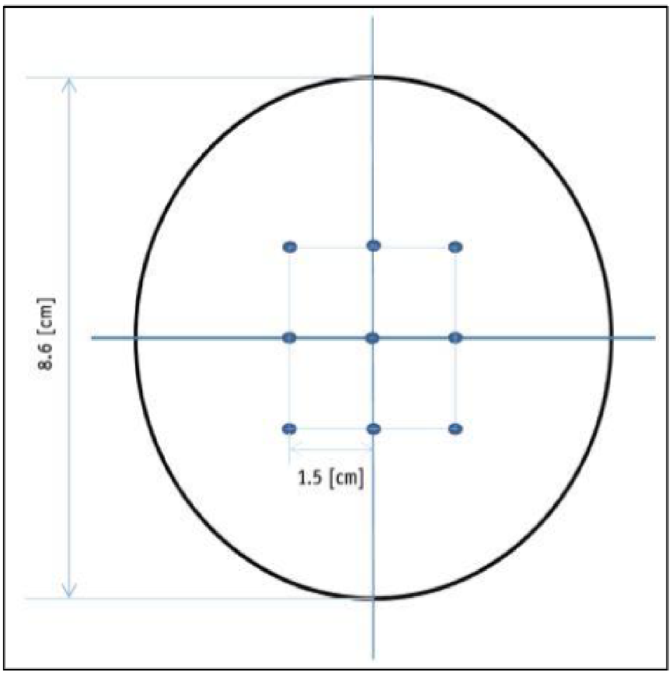

Figure 1: Guide for Seed Placement on Petri Dish

2.4. Simulation

Control: The petri dish labelled G1 was left vertically and 90° and subjected to similar conditions with exception of the treatments. Similarly, G2 was subjected to similar conditions and was placed horizontally. The new gravity parallel vertical line was drawn using a different marker.

Simulated Microgravity: G3 was directly placed on a clinostat using a tape with the vertical orientation maintained prior to clinorotation. Clinorotation began at a pre-selected fast speed of 75rpm and was monitored for every 24 hours (Figure 2) [14].

2.5. Study Parameters

Parameters assessed for the proposed study were short root growth, root length and root curvature. Both root length and curvature of all plants in the experimental and control groups were measured using Image J software.

2.6. Data Analysis

Study outcomes were observed and recorded using Microsoft excel 365, data cleaning and coding was then conducted. The data was then transcribed into SPSS version 25 for statistical analysis. For descriptive statistics, categorical variables were presented in frequency and percentages. Due to the violation of the assumptions of normal distribution, non-parametric test (Kruskal Wallis-H test) was conducted to test for statistical significant differences in seed morphology. Results for continuous variables were presented in median and interquartile range. All statistics was performed considering a 95% confidence level and a 5% level of significance.

Figure 2: 2D Clinostat for Simulation of Microgravity

3. Results

Figure 3 presents an illustration of the outcomes of cowpea seedlings after subjection to 90° positioning, clinorotation and horizontal placement. After 24 hours, visible short root growth was observed of >5 mm in 7 crops each of all groups. A longer root growth was also observed after 48 hours of 90° positioning, clinorotation and horizontal placement. However, the root growth was more in the clinorotated and 90° seedlings compared to those that were placed horizontally (Figure 3).

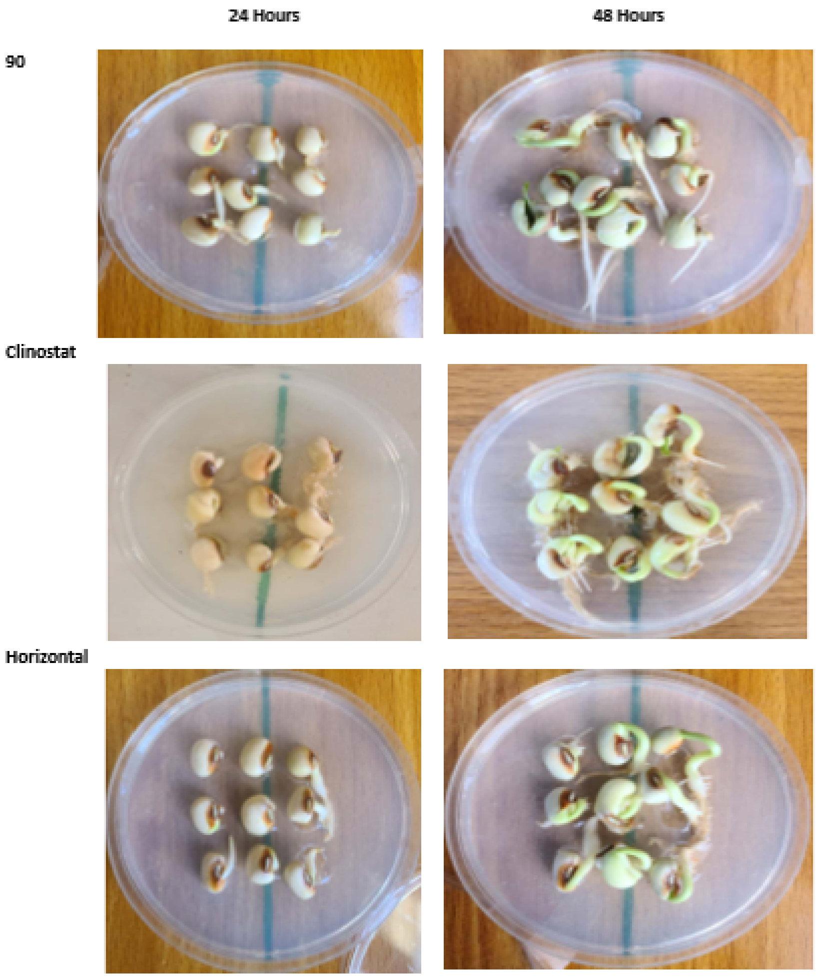

Figure 3: Outcome of Cowpea seedlings after 24 and 48 hours' simulations

Table 2 shows the descriptive statistics of cowpea short root after subjection under clinorotaton, 90° and horizontal positioning. After 24 hours, seven seedlings of all experimental groups had visible short root growth while all nine had visible root growth after 48 hours in all groups.

Table 2: Descriptive Statistics of Cowpea Root Growth after 24 and 48 Hours

Positioning	Visible Growth 24 Hours		Visible Growth 48 Hours	
	Yes	No	Yes	No
90°	7 (77.8)	2 (22.2)	9(100)	0(0.00)
Clinostat	7 (77.8)	2 (22.2)	9(100)	0(0.00)
Horizontal	7 (77.8)	2 (22.2)	9(100)	0(0.00)

Note: °= degrees

As represented in table 3, cowpea subjected to 90° positioning and clinorotation had a median (interquartile range) root growth of 1.86 (1.09) and 1.25 (0.56) compared to seeds that were positioned horizontally [0.94 (1.82)] after 24 hours. After 48 hours, cowpea positioned at 90° vertically had a median (interquartile range) short root growth of 4.45 (2.63) compared to horizontal placement [2.59 (3.59)] and clinorotation [2.78 (0.64)] (Table 3).

Table 3: Median Short Root Growth of Cowpea Seedlings after 24 and 48 hours of experimental simulations

Positioning	Root Growth 24 hours (cm)	Root Growth 48 hours (cm)
	Median (IR)	Median (IR)
90°	1.86 (1.09)	4.45(2.63)
Clinostat	1.25 (0.56)	2.78 (0.64)
Horizontal	0.94 (1.82)	2.59(3.59)

Note: °=degrees; cm=centimeters; IR= interquartile range

The median (interquartile range) curvature of cowpea root was 54.21 (29.15) in those subjected to horizontal placement and 50.84 (58.03) in those clinorotated compared to those of 90° positioning [47.46(19.90)]. However, after 48 hours, cowpea seedlings under clinorotation had median curvature of 69.01 (21.60) compared to those at 90° positioning of 61.75(26.62) and those subjected to horizontal positioning [53.55(9.20)] (Table 4).

Table 4: Curvature of Cowpea Seedlings after 24 and 48 hours of Experimental Simulations

Positioning	Root curvature 24 hours (°)	Root curvature 48 hours (°)
	Median (IR)	Median (IR)
90°	47.46 (19.90)	61.75(26.62)
Clinostat	50.84 (58.03)	69.01 (21.60)
Horizontal	54.21 (29.15)	63.55(9.20)

Note: °=degrees; cm=centimeters; IR= interquartile range

Table 5 represents the statistical difference between the experimental groups and short root growth of cowpea seedlings. After 24 hours, seedlings positioned at 90° had the highest mean rank (14.56) followed by clinorotated seedlings (14.33) compared to horizontally positioned seedlings (13.11). However, there was no statistical significant difference in the median short root growth between the experimental groups (H=0.17, P>.05). Similarly, 90° positioned seedlings had the highest mean rank in the short root growth (18.94) compared to horizontally placed (11.33) and clinorotated (11.72) seedlings after 48 hours. However, this difference was not significantly different between the experimental groups (H=5.25, P>.05) (Table 5).

Table 5: Kruskal-Wallis H test of the Statistical Difference between Experimental Simulations and Short Root Growth of Cowpea Seedlings

Experimental Groups	Mean Rank	Kruskal-Wallis H (df)	P-Value
RG_24 hours		0.17 (2)	.916
90°	14.56		
Clinostat	14.33		
Horizontal	13.11		
RG_48 hours		5.25 (2)	.072
90°	18.94		
Clinostat	11.72		
Horizontal	11.33		

Note: °=degrees; cm=centimeters; RG= root growth; df= degree of freedom; p-value= significance level, hrs=hours

As presented in table 6, plant that were subjected to horizontal placement after 24 hours had the lowest mean rank in the curvature (12.06) compared to clinorotated (14.17) and 90° (15.78). However, this finding was not statistically significant (H=1.05, P>.05). Clinorotated (19.22) seedlings had the highest mean rank compared to 90° (8.61) and horizontal (14.17) and this was statistically significantly different (H=8.14, P<.05) (Table 6).

Table 6: Kruskal-Wallis H test of the Association between Experimental Simulations and Curvature of Cowpea Seedlings

Experimental Groups	Mean Rank	Kruskal-Wallis H (df)	P-Value
Curv_24hrs		1.05 (2)	.592
90°	15.78		
Clinostat	14.17		
Horizontal	12.06		
Curv_48hrs		8.14 (2)	.017
90°	8.61		
Clinostat	19.22		
Horizontal	14.17		

Note: °=degrees; cm=centimeters; Curv= curvature; df= degree of freedom; p-value= significance level, hrs=hours

4. Discussion

The study assessed the development of early root and curvature of cowpea seedlings after being subjected to gravity (horizontal), gravitropic reorientation (90° positioning) and simulated microgravity (clinorotation). Overall, the study demonstrated that simulated microgravity influenced root elongation and curvature after 24 ad 48 hours of observation. However, these differences were not statistically significant.

After 24 hours of observation, visible short root growth was observed in all groups and observed in 7 seedlings in each group. This is consistent findings from a study where after six hours of simulation of microgravity, the short root length was higher compared to seedlings that subjected to 90° positioning [14]. Additionally, all of the seedlings in our study had short root growth after 48 hours. This demonstrates that seedlings flourished under all circumstances and that neither of the experimental settings affected the development of roots. All experimental groups did exhibit root production, but the clinorotated and vertically positioned group had greater root growth that was not statistically significant, according to this study. Furthermore, when seedlings positioned vertically and horizontally were compared to microgravity-simulated seedlings, the direction of root growth was changed. This is in line with studies that have found that growth-related metrics were mostly unaffected by short-term clinorotation, indicating that longer-term studies are required to accurately assess the impact of microgravity on growth parameters [16–19].

The current study found that although insignificant, the root growth for 90° vertically placed and clinorotated cowpea was longer than that of horizontal placed seedlings. A longer root growth may be indicative of better water and nutrient acquisition while promote a firmer anchorage and stability of the plant, resulting in more resilience to environmental conditions. This has been found in studies where plants with longer root growth had higher concentration of minerals like potassium, phosphorus and calcium compared to species with lesser roots [20–22]. Another study found that maize seedlings that were subjected to nitrogen fertilization and had increased root growth had enhanced nutrient uptake efficiency and better shoot growth [23].

According to some theories, the tips of roots are more likely to be enhanced when there is a strong gravitational pull or when there is less gravitational pull than when they are placed horizontally, which causes gravitropic curvature and depends on root elongation and reorientation toward the vector of gravity [3, 18, 24]. Our study found that after 24 hours, the curvature of seedlings in vertically placed and clinorotated groups were lesser compared to horizontally placed seedlings. However, the root curvature became much higher in vertically placed and clinorotated seedlings after 48 hours. While there is no evidence to support this finding, it is suggested that vertically placed seedlings do not have to undergo root reorientation, hence why it should have a smaller curvature compared to horizontally placed seedlings. Our finding also reflects the potential of fluctuation in the growth direction due to the averaging of gravity vector, leading to irregularities in the curvature of the seedlings [25].

This study provides valuable insight to promote long-term space mission and reduce man's dependence on limited supply from earth. However, there were several limitations identified in his study. A relatively small sample size was used which prevented robust analysis and reduced the statistical power. The use of non-parametric test due to violation of assumptions limited he ability to analyze for slight but important biological differences between groups. Also, the low power of the study may have accounted for the insignificant findings identified in our study. Although important, the limitations of this study prevented overstatement of biological differences identified.

This study indicates limited and insignificant effects of gravitational orientation and reorientation on early root growth and curvature of cowpea seedlings. While numerous studies have been conducted to assess the effect of microgravity on seedlings, this study contributes to current knowledge as it was able to assess the morphology of seeds placed on clinorotation and observe early root growth under microgravity as well as other morphological parameters. Although the study found no significant difference in the root growth and curvature of these seedlings, additional research is required to examine other morphological, biochemistry, proximate and nutritional parameters throughout time. Additionally, research using a larger sample size is required to develop representative conclusions.

Article Information

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