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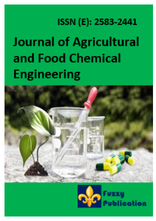

Research Article

Morphologic Features of Zea Mays Subjected to Simulated Microgravity

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Abstract

Background: Giving man's quest for prolonged space mission and the need for sustainable food systems, it is important to study how plant adapt to space conditions such as microgravity. The aim of this study is to examine the morphological difference of *Zea mays* subjected to simulated microgravity. **Methods:** *Zea mays* seedlings were sterilized in a 3% bleach solution for 10 minutes which was followed by rinsing in running water for three times. Seedlings were placed in an Agar Agar solution and randomly assigned to three petri dishes labelled horizontal, 90°, and cinorotation. Clinorotated group was mounted on a clinostat and observed for 24 and 48 hours. Changes in morphology was observed and results analyzed using SPSS. **Result:** After 24 and 48 hours, there was a statistically significant difference in the mean root growth of clinorotated (0.23, 2.31) compared to horizontally (0.00, 0.57) placed seedlings (P<.05). Although mean curvature was lower in clinorotated compared with horizontal and 90° placed groups, this was not statistically significant (P>.05). **Conclusion:** Findings from this study shows that *Zea mays* seedlings adapt to simulated microgravity with better short root growth. However, it is important the other studies are conducted to determine the long-term effect of microgravity on seedlings beyond morphological differences.

1. Introduction

Generally, plants depend on gravitational attraction to enable their organ orientation, growth and development. As a result, plants have evolved to build a mechanism that respond to gravity which allows roots to grow downwards due to positive gravitropism and attach firmly to the ground, and shoot grow upwards through negative gravitropism [1, 2]. In addition to phototropism, these responses control plant growth hormones and influence plant productivity [1]. However, plants adapt when exposed to contrary gravitational conditions, resulting in changes in their growth patterns, cellular structure and other morphological features [3] .

Microgravity has profound impact on plant growth, causing irregularities in shoot and root direction, changes in elongation patterns as well as the structure of the cell wall. However, there have been reports of enhanced germination, leaf expansion and cellular proliferation in

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plants subjected to microgravity [4, 5]. Under this condition, the cell expansion is enhanced which alters the distribution of hormone and stimulates growth responses that allows for efficient use of resources [6]. Findings from numerous studies also suggest that microgravity although alters the pattern of plant growth, can ensure flexibility and adaptive mechanisms that can promote plant growth, development and sustainability in the era where there are concerns about the low production of commercial crops globally [7–9].

Zea mays is one of the most frequently grown grains in the world. The cultivation of this commercial cereal grain started in Central America and has since spread across agro-ecological zones globally [10]. It is one of the most adaptable and economically significant crops, playing a crucial role in global food security and agricultural sustainability [11]. The demand for *Zea mays* continues to increase globally due to population growth, urbanization, and industrial expansion, focusing on sustained improvement in productivity and resilience [12]. Cultivation of *Zea mays* includes several phases such as seed selection, land preparation, planting, germination, seedling establishment, vegetative growth, reproductive development, and harvesting [13]. Factors such as soil fertility, moisture availability, temperature, and pest pressure influence the duration and success of each stage [13].

Despite the large production volume and favorable agro-ecological conditions in Nigeria, maize yield remains lower than its potential yield, with an estimated annual supply gap of around 4 million tonnes [14]. This yield gap is caused by factors such as low soil fertility, limited use of improved seed, post-harvest losses, pest and disease outbreaks, high input costs, low rainfall, lack of agronomic practice and mycotoxins caused by fungi during storage which reduce quality and profitability [13]. These factors collectively limit productivity and food security. Under microgravity conditions, plants exhibits enhanced physiological and structural traits. Studies have shown that *Zea mays* subjected to microgravity conditions exhibits improved nutrient utilization and more uniform root nutrient distribution, which could boost the development of high-yield and provide efficient crops suitable for both earth and space farming [15, 16]. Microgravity also enhances biomolecule production and alters gravitropic signal allowing researchers to identify genes and hormones responsible for growth direction and structure [17]. However, previous studies allowed for short root growth prior to mounting and observation of the effect of clinorotation making it difficult to fully attribute findings to the direct simulation. Thus, our study examines the effect of direct clinorotation on the morphology of *Zea mays* in the absence of prior short root growth.

2. Method

Experimental Design

An experiment was conducted in the Life Science laboratory of the National Space Research and Development Agency from November 7–9, 2025 to determine the morphological differences in clinorotated *Zea mays. Zea Mays* was commercially purchased from a farm in Piwoyi, Federal Capital Territory (FCT), Nigeria.

Materials and Equipment

Materials included in this study were *Zea mays* (the same cultivar, age and size), petri dish, agar agar nutrient medium, 5% bleaching agent (sodium hypochlorite), glass rod, forceps, wetting agent, paper towels, transparent stick film, permanent markers, heating plate, pH strip, thermometer, plastic box (wet chamber), weighing scale, hygrometer, camera, two-dimension clinostat, wooden holders, measuring tools (caliper, digital camera, ruler, analytic balance), data management and analytic tool (laptop, Microsoft Excel, SPSS).

Study Procedure and Assignment

Bleaching

A 3% measure of bleach solution was prepared as demonstrated below;

3% bleach solution = 3ml Bleach: 97ml distilled water

This bleach solution was prepared to sterilized seedlings. Maize seedlings were soaked in this solution for 10 minutes and rinsed off 3-5 times with running tap water. The seeds were randomly assigned to groups *(Zm1, Zm2, and Zm3)*.

Preparation of Growth Medium

One liter of the medium was prepared by adding 3g of agar-agar powder into a 2 liter of tap water in a beaker. Using a glass rod. the mixture was stirred thoroughly until well combined. The beaker containing the mixture was heated on a hot plate under low heat while continuously stirring until the mixture wass completely dissolved providing a clear solution [18]. A pH strip was used to measure the pH of the solution and maintain at 5. After resting for five minutes, the solution was transferred into each sterile petri dish (*Zm1, Zm2, Zm3*) to sit at room temperature [18]. These mediums will be labelled *Zm1, Zm2, and Zm3* Table 1.

Table 1: Assignment of Seedlings into Experimental Groups

Group	Treatment
Zm1	Control (horizontal)
Zm2	Control (vertically inverted at 90°)
Zm3	Clinorotated

Note: G=Group

All petri dishes were mounted on a template where a vertical reference line and drawn to indicate the direction of gravity [18]. Nine seedlings were placed in the same direction in each petri dish Figure 1. After placement, the petri dish was covered with a lid and sealed with a transparent stick films with gaps for oxygen support. These petri dishes were assigned to their experimental groups for experimentation and the effect of clinorotation on germination and seed morphology was observed. *Zm1* was placed vertically on a wooden holder at 90°, and *Zm2* horizontally in a wet chamber with 40cm x 40cm x 40cm dimension. The humidity of the chamber was maintained between 60-100% [18]. Observation was done for 24-48 hours for visible short root growth (5mm-10mm) and morphological differences [18].

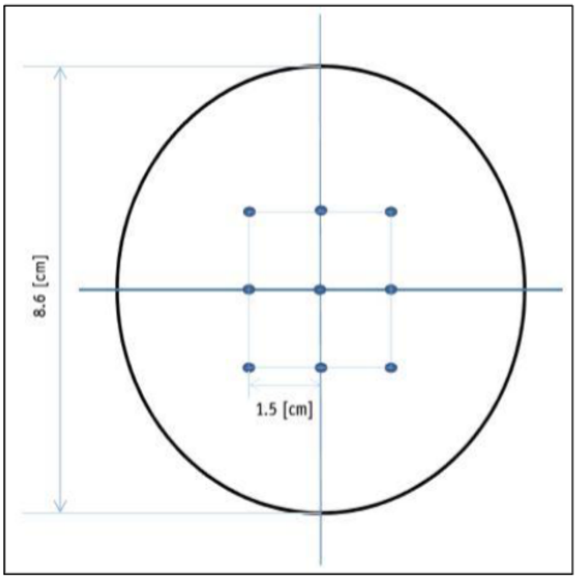

Figure 1: Guide for Seed Placement on Petri Dish

Simulation

Control: *Zm1* was vertically placed at 90° and subjected to similar conditions except for treatments. *Zm2* was placed horizontally and similarly subjected the same conditions. The new gravity parallel vertical line was drawn using a different marker.

Microgravity: *Zm3* was positioned on a clinostat using transparent stick tape while maintaining the vertical orientation prior to inducement. Clinorotation commenced using a pre-selected speed of 75rpm and the procedure was monitored for every 24 hours for 48 hours.

Study Parameters

Short root growth, root length and root curvature were the parameters assessed for the proposed study.

Data Management

Data collection, cleaning and coding was conducted using Microsoft excel 365. Coded data was then transcribed into SPSS version 25 for statistical analysis. Descriptive statistics was presented as frequency and percent for categorical variables. Assumption for normality was violated leading to the use of non-parametric test (Kruskal Wallis-H test) to test for statistical significant differences in seed morphology. Thus, descriptive statistics for continuous variables were presented in median and interquartile range. The statistics was conducted using a 95% confidence level and 5% level of significance.

3. Results

Table 2 presents the descriptive statistics for visible root growth after 24 and 48 hours of observation. After 24 hours, no seedling was horizontally positioned had visible root growth while only 1(11.1) of the seedling placed at 90° had visible root. Also, 5(55.6) of the clinorotated seedlings had visible root growth. After 48 hours, 7(77.8) of the seedlings in all group had visible root growth while 2(22.2) had no visible growth Table 2.

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

Positioning	**Visible Growth 24 Hours**		**Visible Growth 48 Hours**	
	Yes	No	Yes	No
90°	1(11.1)	8(88.9)	7(77.8)	2(2.2)
Clinostat	5(55.6)	4(44.4)	7(77.8)	2(2.2)
Horizontal	0(0.0)	9(100)	7(77.8)	2(2.2)

Note: °=degree

As presented in Table 3, the mean root growth of 90° placed after 24 hours was 0.04±0.12 while clinorotated seedlings had mean root growth of 0.23±0.28. After 48 hours, the mean root growth was lower in horizontally placed seedlings (0.57±0.48) compared to those clinorotated (2.31±1.57) and 90° positioned (1.98±1.56) Table 3.

Table 3: Mean Root Growth of Maize Seedlings after 24 and 48 Hours of Observation

Positioning	Root Growth 24 hours (cm)		Root Growth 48 hours (cm)	
	Mean ± SD	95% CI	Mean ± SD	95% CI
90°	0.04 ± 0.12	-0.05 – 0.13	1.98 ± 1.56	0.78 – 3.19
Clinostat	0.23 ± 0.28	0.02 – 0.44	2.31 ± 1.57	1.11 – 3.51
Horizontal	0.00 ± 0.00	0.00 – 0.00	0.57 ± 0.48	0.20 – 0.95

Note: °=degree; cm=centimeter; SD=standard deviation; CI=confidence interval

After 48 hours, the mean curvature of maize seedlings was higher in seedlings positioned at 90° (44.83 ±34.82) compared to clinorotated (17.68±35.10) and seedlings horizontally positioned (40.17±31.47) Table 4.

Table 4: Mean Root Curvature of Maize Seedling After 24 and 48 Hours of Observation

Positioning	Curvature 24 hours (°)		Curvature 48 hours (°)	
	Mean ± SD	95% CI	Mean ± SD	95% CI
90°	0.00 ± .000	0.00 – 0.00	44..83 ±34.82	18.06 – 71.59
Clinostat	0.00 ± 0.00	0.00-0.00	17.68 ± 35.10	-9.30 – 44.66
Horizontal	0.00 ± 0.00	0.00-0.00	40.17 ± 31.47	15.99 – 64.36

Note: °=degree; SD=standard deviation; CI=confidence interval

A presented in Table 5, there was a statistically significant difference in the mean root growth of maize seedlings for the three treatments [F (2, 24) = 4.46, P: .023] after 24 hours of observation. After 48 hours, there was a statistically significant mean root growth in the three groups [F (2, 24) = 4.48, P:.022] Table 5.

Table 5: One-Way ANOVA of the Statistical Difference between the Mean Root Growth of Maize Seedling Subjected to Clinorotation and Horizontal and 90° Placement

		Sum of Squares	Df	Mean Square	F	P value
RG_24hrs	**Between Groups**	.266	2	.133	4.463	.023
	Within Groups	.717	24	.030		
	Total	.983	26			
RG_48hrs	**Between Groups**	15.343	2	7.671	4.480	.022
	Within Groups	41.094	24	1.712		
	Total	56.436	26			

Note: RG=root growth; °=degree; df= degree of freedom; cm=centimeter, SD=standard deviation, CI=confidence interval; F= F test

Table 6 presents the post-hoc analysis of the differences of the mean root growth between the three groups. There was a statistically significant difference in the mean root growth between horizontally placed compared to clinorotated maize seedlings (d=0.23, P;.026). Similarly, there was a statistically significant difference in the mean root of maize seedlings subjected to clinorotated compared to horizontally placed seedlings (d=1.74, P:.025) Table 6.

Table 6: Post-Hoc Analysis of the Difference between the Mean Root Growth of Maize Seedling Subjected to Clinorotation and Horizontal and 90° Placement

Dependent Variable	Rep_Ct	Rep_Ct	Mean Difference	P value	95% CI
RG_24hrs	90	Clinostat	-.19	.073	-0.39 – 0.01
		Horizontal	.04	.881	-0.16 – 0.24
	Clinostat	90	.19	.073	-.01 – 0.39
		Horizontal	.23*	.026*	.02 – 0.43
	Horizontal	90	-.04	.881	-.24 – 0.16
		Clinostat	-.23*	.026*	-.43 - -0.02
RG_48hrs	90	Clinostat	-.33	.859	-1.87 – 1.21
		Horizontal	1.41	.077	-.13 – 2.95
	Clinostat	90	.33	.859	-1.21 – 1.87
		Horizontal	1.74*	.025*	.20 – 3.28
	Horizontal	90	-1.41	.077	-2.95 – 0.13
		Clinostat	-1.74*	.025*	-3.27 - -0.20

Note: RG=root growth; °=degree; cm=centimeter, SD=standard deviation, CI=confidence interval

As presented in Table 7, there was no statistically significant difference in the mean root curvature of maize seedlings in the three groups [F (2, 24) = 1.66, P:.212].

Table 7: One-Way ANOVA of the Difference between the Mean Root Curvature of Maize Seedling Subjected to Clinorotation and Horizontal and 90° Placement

		Sum of Squares	df	Mean Square	F	Sig.
Cur_48hrs	**Between Groups**	3792.99	2	1896.49	1.66	.212
	Within Groups	27473.18	24	1144.72		
	Total	31266.17	26			

Note: df= degree of freedom; Curv=curvature; F= F test

4. Discussion

This study evaluated the early morphological response of *Zea mays* seedlings subjected to direct clinostat rotation, with keen interest to germination rate, root elongation, and curvature. The results demonstrate that simulated microgravity significantly influences early seedling development, and this shows both agreement and deviation from previously published related studies.

The higher percentage of visible root growth observed in the clinorotated group within the first 24 hours corroborated by earlier findings that microgravity improves germination and radicle protrusion. Studies have also reported that microgravity elevates water uptake, enzyme activation, and early radicle emergence due to reduced mechanical resistance and altered hormone gradients [6, 19–21]. The present result is consistent with this, as 55.6% of clinorotated seedlings showed visible roots at 24 hours compared to only 11.1% in the 90° group and none in the horizontal group. This confirms the claim by previous authors that microgravity increases first physiological events related to germination [20, 22].

Elongation of the root was significantly higher as observed in clinorotated seedlings after 48 hours, which is consistent with studies that reported improved cell expansion and increase in elongation of seed roots under simulated microgravity [6, 23]. Studies conducted on maize and other cereals, shows that microgravity redistributes auxin more evenly and reduces differential growth pressures, thereby allowing roots to elongate rapidly [24–26]. Furthermore, comparable studies on Arabidopsis and wheat also recorded increased root extension under microgravity simulation through clinorotation due to reduced gravitropic constraint [27, 28]. This demonstrates that our findings support a widely accepted pattern in plant–microgravity research. However, the least root elongation was observed in the horizontal orientation, which has similarity with previous work showing that misalignment of the natural gravity vector disrupts gravitropic sensing and delays radicle penetration [29]. Horizontally oriented roots are exposed to conflicting gravitropic signals, which interferes with directional growth and slows elongation [1, 3].

In contrast to many earlier studies, root curvature did not differ significantly between the three treatments in our experiment. Previous microgravity studies often record reduced or irregular root curvature due to the absence of a stable gravity vector [3, 30]. One of these studies revealed a suppression in normal gravitropism due to clinorotation, producing measurable curvature deviations [30]. The present study showed that clinorotated roots had lower curvature compared to the 90° and horizontal controls, but the difference was not statistically significant. One notable methodological difference is that those earlier studies allowed seedlings to develop short roots before mounting them on the clinostat, while the present study exposed seeds to microgravity from the onset of germination. This methodological difference may explain the weaker curvature effects here and indicates that the timing of exposure to microgravity has a major influence on gravitropic signaling.

The current study found significant differences in the mean root length in the clinorotated compared to horizontal group with similar findings found in other studies [20, 26, 31]. One of these studies attributes the differences in root morphology to the alteration in gravity perception and the certain adaptations to the physiology responsible for plant growth [31]. Also, another study reported alteration in the organization of cortical microtubules which causes autophagy and cytoskeletal related modification of gene expression which affects cellular and morphological dynamics of clinorotated plants [32]. This suggest that while the direction-sensing of the clinorotated crops are altered, there is a higher tendency of adaptation and improvement in overall growth especially when clinorotation occurs at early developmental stages. Thus, *Zea mays* may respond positively to microgravity and could be suitable for exploring cultivation in extraterrestrial environments.

This study has established the enhancement of germination and elongation of *Zea mays* in response to clinorotation. This finding contributes to the suggestion that *Zea mays* possesses good adaptation and sustainability to extreme environmental conditions while reiterating the need for explore more exposure timing in further studies. As reflected in the image, clinorotated *Zea mays* seedlings produced multiple root growths per seed with lesser organization in root architecture. This phenomenon is proposed to have been as a result of disruption in gravity sensing and the alteration in auxin distribution [18]. Under normal gravity, statolith settling creates a stable auxin gradient that directs elongation into a single dominant primary root. However, when this gravity vector is randomized through clinorotation, auxin becomes more diffusely distributed, often times leading to ectopic initiation of root primordia and the formation of multiple sprouts [19, 20].

Article Information

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Ethical Approval: Ethical approval was not required for this study because it did not involve human participants, identifiable human data, or animals.

Informed Consent: Not applicable.

Conflict of Interest: The authors declare no competing interests.

Data Availability Statement: Data availability is not applicable to this article because no datasets were generated or analyzed.

Disclaimer (Artificial Intelligence): The author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.), and text-to-image generators have been used during writing or editing of manuscripts.

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