Short-term Morphologic Differences of Soya Bean Subjected to Microgravity

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Abstract:

➢ ***Background*:**

It is important to understand plant responses to altered gravity as it is important to advance space agriculture and improve food sustsainability. The effect of microgravity may elicit significant physiologic and morphologic changes that can be important to the economic growth of soya bean seedlings. This study assessed the morphological effects of simulated microgravity on soya bean seedling.

➢ ***Method:***

Soya bean seedlings was germinated on agar medium on a petri dish and were assigned to horizontal, 90 ° orientation and clinorotation groups. Root growth, length and curvature were monitored after 24 and 48 hours. Data was collected and analyzed using SPSS

➢ ***Results:***

After 24 hours, root length of clinorotated (0.75cm) seedlings was higher compared to those vertically (0.58cm) and horizontally (0.50cm) controls after 24 hours ($P<.05$). However, vertical group (2.52cm) had the highest root growth after 48 hours ($P<.05$). The root curvature was higher in the clinorotated (27.11°) and horizontally (29.75°) placed seedlings after 24 hours. After 48 hours, the vertically (33.41°) and horizontally (40.51°) placed seedlings had higher curvature compared to the clinorotated (26.14) group but this was not statistically significant ($P>.05$).

➢ ***Conclusion:***

This study finding reveals that short-term clinorotation can enhance early root growth. The study elucidates early plant development with altered gravity and provides insight necessary for space biology and improving agriculture.

Keywords: *Soya Bean, Microgravity, Clinostat, Clinorotation, Glycine Max.*

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I. INTRODUCTION

The curiosity in understanding the responses elicited by plant cells physiologically and biochemically under anecdotal conditions have been explored recently, using ground-based experiments to simulate space technology. Hence, it is crucial to obtain an inclusive understanding of the processes required in plant growth and improvements under microgravity conditions [1].

Gravitational force is responsible for the weight of an object in planetary surface. Objects on earth gravitational force experience acceleration of 9.81 m/s^2 pull to the center of the planet and it is usually denoted as (1g' Gravity) [2]. Microgravity in the other-hand is an outstanding environment that elicits transformation of both the physical and physiological features of plants [3]. These changes make it necessary for a thorough study of plant growth and development in space agriculture. Because space travel is restricted and cost intensive, gyratory contraptions instruments such as clinostat and Radom Positioning Machine (RPM) have been used to mimic microgravity on Earth. Light microgravity exposure on seedlings has also been implicated as a key factor for its germination and maturation [4].

A deep incite on the impact of microgravity on seed germination, seed growth, development, and overall plant advancement is critical for prospective space farming and the advancement of sustainable food production systems [5]. Soya bean (*Glycine max*) is one of the essential staples, rich in protein and is chiefly the leguminous plant which contains high amounts of essential omega-3 fatty acid and alpha-linolenic acids [6]. Additionally, soya bean contains biotin, a vital nutrient factor in higher concentration compared to vegetables and fruits respectively [7].

Soybean is a remarkably adaptable crop and is cultivated on over 50 million hectares which si translated to about 35% of the world's vegetable oil is produced from soyabeans [7]. The Asians depend majorly on the protein produced from the staple [8]. Due to its importance, much emphasis has been made globally on ways of improving the crop genetically [9]. It has been reported that breeding of the crop has been on for over sixty years in the North America and continues in other parts of the world [10]. More works on soyabeans via microgravity simulation will give more insight on the physiological and biological make-up of the seedlings. Therefore, the aim of the study is to examine the morphological effects of soya bean seedlings subjected to simulated microgravity.

II. METHOD

- *Experimental Design*

Soya bean seedlings (Tax1448-2E) was purchased from International Institute of Tropical Agriculture, Federal Capital Territory (FCT), Nigeria.

- *Study Procedure and Assignment*

About 3% of bleach solution was prepared to sterilized seedlings. The Seeds (cowpea) were soaked in this solution for 10 minutes and rinsed off 3-5 times with running tap water. The seeds were randomly assigned to three groups. About 3g of agar-agar powder was added into a two liters of tap water in a beaker and the mixture was stirred using a glass rod. The solution was heated on a hot plate and continuously stirred until a clear solution was observed [11]. Using a pH meter, the pH of the solution was measured at 5 and this solution was let to rest for five minutes. The agar solution was transferred into each three sterile petri dish and let to sit at room temperature [11]. One petri dish was labelled as horizontal control and the other vertical at 90⁰ while the third group was labelled the clinorotated group.

- *Experimentation*

About 9 soya bean seedlings were placed in each petri dish and a vertical reference line was drawn indicating the direction of gravity as explained in the teacher manual [11]. The petri dish was covered with a lid and transparent stick films to seal the petri dishes with gaps for oxygen support. One of the petri dishes in the control was placed vertically on a wooden holder at 90º, and the other horizontally in a wet chamber (40cm x 40cm x 40cm dimension). The humidity of the chamber was maintained at 75% as specified by the teachers guideline [11]. The seedlings were monitored for 24-48 hours for visible short root growth (5mm-10mm) and the changes in root length and curvature [11] (Figure 1).

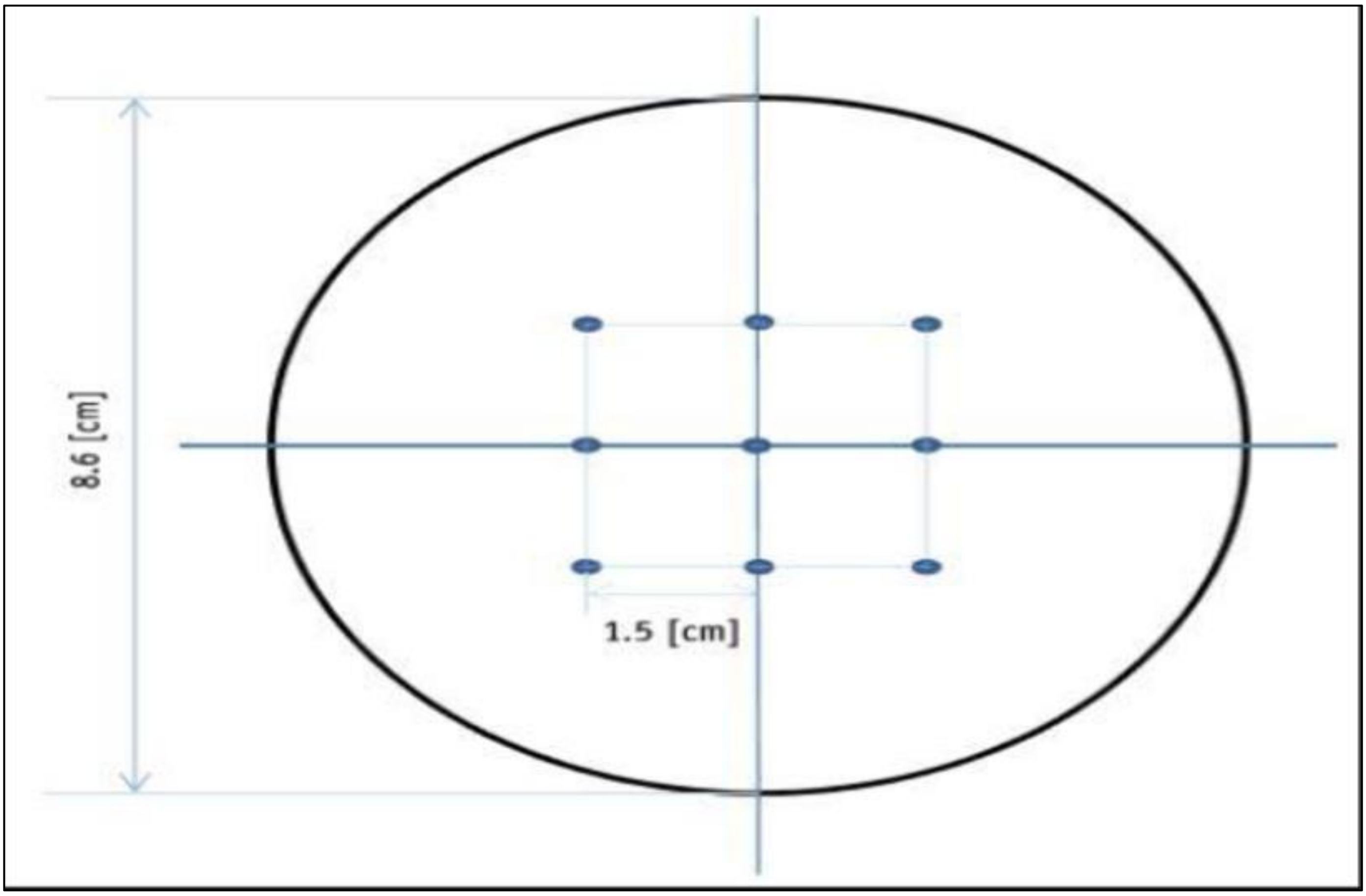

Fig 1 Seed Placement Guide for Microgravity Experiment

The microgravity group was directly placed on a clinostat using a tape with the vertical orientation was maintained before clinorotation at a pre-selected speed of 75rpm. The clinorotated seedlings were monitored for 24 and 48 hours (Figure 2). All petri dishes were subjected to similar conditions with exception of the treatment.

Fig 2 Two-Dimensional Clinostat

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➢ *Study Parameters*

Data collected include visible short root growth, mean root length and curvature.

➢ *Data Analysis*

Data was collected using Microsoft excel 365 where data cleaning and coding were performed and SPSS version 25 was used to conduct statistical analysis. Descriptive statistics was presented in frequency and percentage categorical variables. One-way Analysis of Variance (ANOVA) was used to determine the statistical differences in mean root length and curvature between treatment groups. Statistics was performed at a 95% confidence level and 5% level of significance.

III. RESULTS

Figure 3 presents the outcomes of soya bean seedlings after 24 and 48 hours of simulation. While there was visible shoot root growth observed in some of the seedlings after 24 hours of observation, these roots were relatively small compared to 48 hours of which those that were placed vertically at 90º and clinorotated had longer root growth compared those that were horizontally placed. Also, those that were placed vertically were found to have a gravitropic root growth compared to those that were horizontally placed and clinorotated which seems to be plagiotropic (Figure 3).

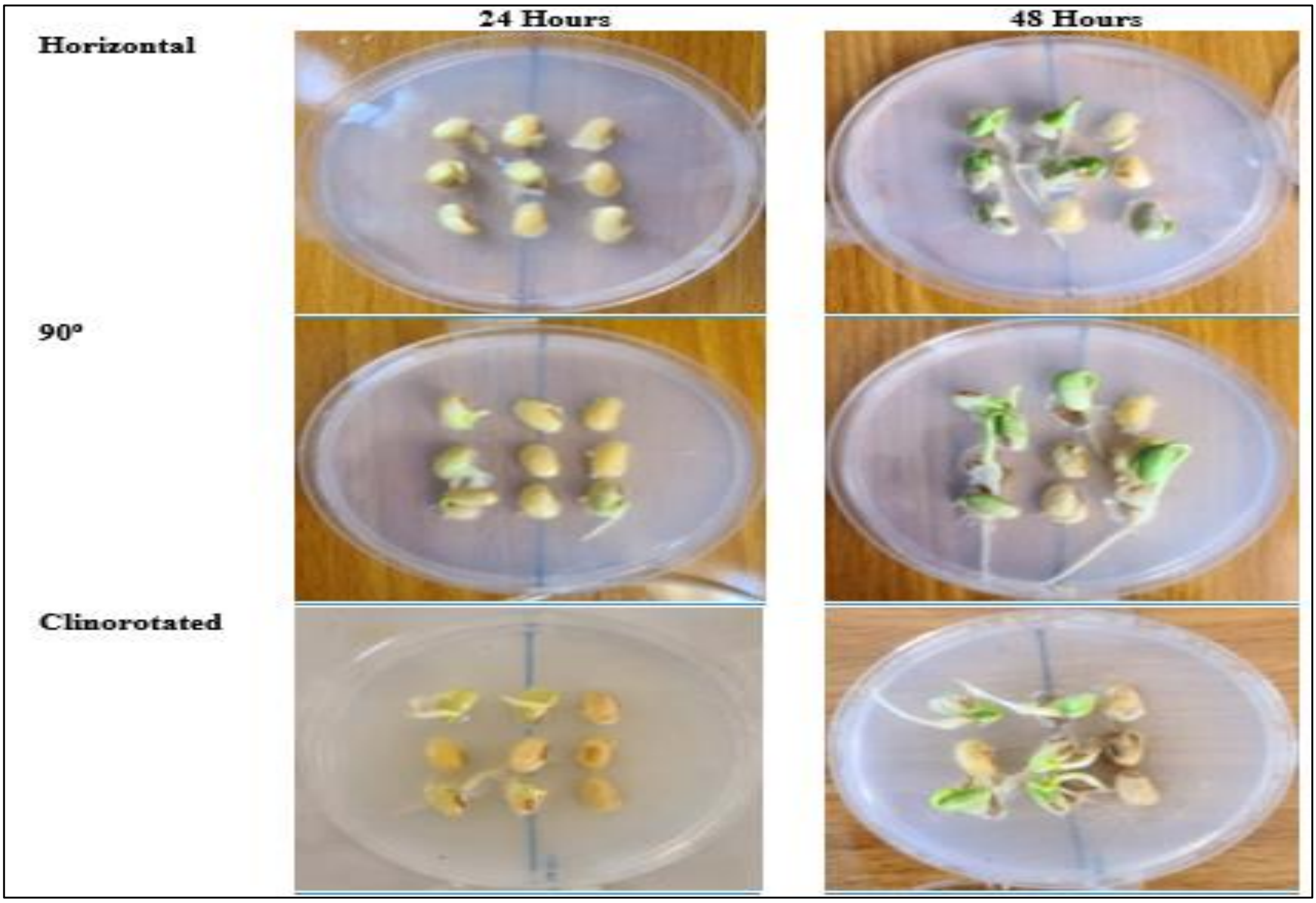

Fig 3 Soya Bean Seedling Outcomes after 24 and 48 Hours of Experimentation

As presented in Table 1 visible root growth was observed in 44.4% of the seedlings that were vertically placed at 90º and those that were horizontally placed. However, 55.6% of the clinorotated seedlings had visible root growth. After 48 hours, 77.8% of the horizontally placed seedlings had visible root growth compared to the vertically placed seedlings where 55.6% of the seedlings had visible growth. The number of seedlings with root growth did not change in those that were clinorotated after 48 hours (Table 1).

Table 1 Descriptive Statistics of Visible Root Growth After 24 and 48 Hours of Experimentation

Positioning	**Visible Growth 24 Hours**		**Visible Growth 48 Hours**	
	Yes	**No**	**Yes**	**No**
90º	4(44.4)	5(55.6)	5(55.6)	4(44.4)
Clinostat	5(55.6)	4(44.4)	5(55.6)	4(44.4)
Horizontal	4(44.4)	5(55.6)	7(77.8)	2(22.2)

Note: º=Degrees

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The mean root length of clinorotated seedlings (0.75cm) was higher compared to horizontally (0.50cm) and vertically (0.58cm) placed seedlings after 24 hours. However, the mean root length after 48 hours was higher in vertically (2.52cm) and horizontally (1.97cm) placed seedlings compared to those that were clinorotated (1.50cm) (Table 2).

Table 2 Statistics of the Mean Root Length of Soya Bean Seedlings after 24 and 48 Hours of Experimentation

Positioning	Root length 24 hours (cm)		Root Length 48 hours (cm)	
	Mean ± SD	95% CI	Mean ± SD	95% CI
90°	0.58±0.83	-0.06-1.22	2.52±3.18	0.08-4.97
Clinostat	0.75±0.83	0.11-1.39	1.50±1.55	0.31-2.69
Horizontal	0.50±0.70	-0.03-1.04	1.97±2.07	0.38-3.57

Note: °=Degrees; cm=Centimeters; SD=Standard Deviation; %=Percent; CI=Confidence Interval

Table 3 presents the analysis of the mean differences between experimental groups and root length of soya bean seedlings. There was a statistically significant difference in the mean root growth of soya bean seedlings between the three treatments after 24 hours of exposure to experimentation [F (2, 10) = 74.26, *P: .000*]. After 48 hours, there was also a statistically significant difference in the mean root growth of the three groups [F (2, 10) = 13.26, *P:.040*] (Table 3).

Table 3: One-Way ANOVA of the Statistical Difference Between the Mean Root Length of Soya Bean Seedling Subjected to Clinorotation and Horizontal and 90° Placement

		Sum of Squares	Df	Mean Square	F	P value
RL_24hours	Between Groups	1.06	2	0.53	74.26	*.000*
	Within Groups	0.07	10	0.01		
	Total	1.13	12			
RL_48hours	Between Groups	26.51	2	13.26	4.10	*.040*
	Within Groups	45.30	14	3.24		
	Total	71.81	16			

Note: RL=Root Length; Df=Degree of Freedom

Table 4 presents the post-hoc analysis of the differences in the mean root length after 24 and 48 hours. There was a statistically significant difference in the mean root length between clinorotated seedlings compared to those placed at 90° (d=0.59, *P;.000*) after 24 hours of experimentation. Similarly, there was a statistically significant difference in the mean root of soya bean seedlings that were placed at 90° compared to horizontally placed seedlings after 48 hours (d=2.81, *P:.046*) (Table 4).

Table 4: Post-Hoc Analysis of the Difference Between the Mean Root Length of Soya Bean Seedling Subjected to Clinorotation and Horizontal and 90° Placement after 24 Hours

Dependent Variable	Group	Group	Mean Difference	P value	95% CI
RL_24 hours	90°	Clinostat	-.59	.000	-.74--.43
		Horizontal	.00	.997	-.17-0.16
	Clinostat	90°	0.59	.000	0.43-0.74
		Horizontal	0.58	.000	0.43-0.74
	Horizontal	90°	0.00	.997	-.16--.43
		Clinostat	-.58	.000	-.74--.43
RL_48hours	90°	Clinostat	2.64	.086	-.34-5.62
		Horizontal	2.81	.046	.05-5.56
	Clinostat	90°	-2.64	.086	-5.62-0.34
		Horizontal	0.17	.986	-2.59-2.92
	Horizontal	90°	-2.81	.046	-5.56--.05
		Clinostat	-0.17	.986	-2.92-2.59

Note: RL=Root Length; °=Degree; CI=Confidence Interval

Table 5 presents the curvature of seedling root after 24 and 48 hours' observation in the three groups. The mean curvature was higher in clinorotated (29.75°) and horizontally (27.11°) placed seedlings compared to those that were vertically placed at 90° (20.59°) after 24 hours. After 48 hours, the mean curvature was higher in horizontally (40.51°) and vertically (33.41°) placed seedlings compared to those that were clinorotated (26.14°) (Table 5).

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Table 5 Statistics of Mean Curvature of Soya Bean Root Curvature of 24 and 48 Hours of Experimentation

Positioning	**Curvature 24 hours (°)**		**Curvature 48 hours (°)**	
	Mean ± SD	**95% CI**	**Mean ± SD**	**95% CI**
90°	20.59±26.61	0.14 –41.04	33.41±41.80	1.28–65.55
Clinostat	27.11±33.52	1.34–52.88	26.14±33.69	0.25–52.04
Horizontal	29.75±41.80	2.48–57.02	40.51±38.57	10.86–70.16

Note: °=Degrees, CI=Confidence Interval; %=percent; SD=Standard Deviation

As presented in table 6, there was no statistically significant difference in the mean root curvature of soya bean seedlings in the three groups after 24 hours [F (2, 9) = 2.35, *P:.151*]. After 48 hours, the difference in the mean curvature of soya bean seedlings was not statistically significant between the three experimental groups [F (2, 9) = 2.31, *P:.121*] (Table 6)

Table 6: One-Way ANOVA of the Difference Between the Mean Root Curvature of Soya Bean Seedlings Subjected to Clinorotation and Horizontal and 90° Placement

		Sum of Squares	**Df**	**Mean Square**	**F**	**P value**
RC_24hours	Between Groups	899.73	2	449.86	2.35	.151
	Within Groups	1724.79	9	191.64		
	Total	2624.52	11			
RC_48hours	Between Groups	3165.46	2	1582.73	2.31	.121
	Within Groups	16437.09	24	684.88		
	Total	19602.54	26			

Note: RL=Root Curvature; Df=Degree of Freedom

IV. DISCUSSION

In this study, we investigated the morphological effects of clinorotation on early root growth, root length and curvature of soya bean seedlings. The outcome of the experiment reveals that the subjection of these seedlings to the various exposures after 48 hours of exposure significantly influenced the growth parameters.

As observed in our study, the mean root length was significantly higher in clinorotated seedlings compared to the vertically and horizontally placed seedlings. Similar findings have been identified in a study on Arabidopsis seedlings where the root length and growth rate was significantly higher in clinorotated seedlings compared to controls [4]. Although the 24 hours' time point was not assessed, another study on soya bean seedlings found that root growth under clinorotation was 125% higher compared to vertical controls [12]. However, the root length was significant longer in and vertically and horizontally placed compared to clinorotated seedlings. This transient enhancement could be as a result of the elimination of directional gravitotropic signals and the redistribution of auxins [4,13]. Another reason for this transient elongation can be seen in the number of seedlings that had visible root growth after 24 hours which was higher in clinorotated seedlings compared to other groups. However, prolonged exposure to stress form clionorotation may impair auxin transport which can compromise root elongation after 48 hours of exposure [14]. Our finding suggest that gravity may potentially constrain root elongation at the early phase but long-term plant growth may require a stable gravitropic cue supporting the role of gravitropism developmental adaptivity and not just orientation [15]. Thus, changes in gravity conditions may alter the distribution of auxin and result in disruption in the proliferation of root meristem which can affect the pattern in root growth [16,17]. There is also the need to explore if early, short-term exposure to microgravity can potentially enhance auxin distribution and growth patterns of plant seedlings.

The current study also provided insight on how clinorotation can influence root curvature. As observed in other studies where early gravitropic response as seen in reorientation of gravity vector, our study found that seedlings subjected to clinorotation and horizontal placement had higher curvature compared to those placed at 90° after 24 hours [18]. Another study found that there was significant increase in the root curvature of seedlings subjected to clinorotation with increase in time of clinorotation [19]. Similar to a previous study, the root curvature of clinorotated seedlings was lesser compared to those placed at 90° and horizontally placed after 48 hours [20]. While horizontally positioned seedlings displays consistent increase in curvature as a result of sustained gravitropism, seedlings subjected to simulated microgravity may exhibit secondary curvature response during root elongation [21]. The absence of statistical difference in root curvature suggests the potential of seedlings to acclimatize to simulated microgravity over prolong time of minimal gravity vector.

In summary, the current study reveals that while root growth is improved with short-term subjection to simulated microgravity the curvature was negatively affected. However, these effects observed were transient and there is a potential of seedlings to adjust to extreme conditions over time. One strength of this study is we used clinorotation which is controlled and an established method for simulating microgravity and to allow for comparison with vertically and horizontally placed seedlings. We also used three different orientations which allowed us to evaluate growth response to gravity and microgravity. Despite the contributions, this study was conducted in a relatively short duration which

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prevented us from observing long-term developmental effects. Also, the sample size was relatively small which reduced statistical power. The study only assessed morphological differences and did not assess physiological and molecular parameters. Therefore, future studies should be conducted to explore other effects beyond morphological differences and extend duration of simulation. Also, other studies should increase sample size and incorporate more time points of observation to improve statistical power and robustness. It is also important to assess how short-term auxin redistribution and root elongation can be translated in space biology and improve agriculture in a long run.

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