



Centre for Atmospheric Research

# 2018

## MONOGRAPH OF ATMOSPHERIC RESEARCH

Edited by A.B. Rabiou and O. E. Abiye

A Publication of  
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## PREFACE

The Centre for Atmospheric Research was established in January 2013 with a compelling mission to improve our understanding of the behaviour of the entire spectrum of the Earth's atmosphere; promote capacity development in relevant atmospheric sciences as a way of facilitating international competitiveness in research being conducted by atmospheric scientists; and disseminate atmospheric data/products to users towards socio-economic development of the Nation. CAR's extant core research focus includes: space weather, tropospheric studies, atmospheric research software and instrumentation development, microgravity and human space technology, and atmospheric chemistry and environmental research.

Pursuant to the above, The *Monograph of Atmospheric Research* published by the Centre for Atmospheric Research (CAR), is a collection of peer-reviewed manuscripts in Atmospheric Sciences and closely related fields. This maiden edition comprises articles presented during two separate workshops; *1<sup>st</sup> National Workshop on Microgravity and Environmental Research* (26 - 29 November, 2017) and *1<sup>st</sup> National Workshop on Air Quality* (13 - 16 March, 2018). Such workshops are integral part of CAR's capacity building program and they were primarily aimed at advancing the course of atmospheric research in Nigeria towards sustainable development. The Microgravity workshop was geared towards introducing new research opportunities in space life science by simulating microgravity conditions here at the earth's surface as a means of investigation space biological environment. The Air Quality workshop was organized in collaboration with Ministry of Environment and Nigerian Meteorological Agency (NIMET). The workshop analysed current Air Quality scenario in Nigeria, explored new opportunities for collaborative research and offered novel means of improving the present quality of life of the populace without jeopardizing the chance of the future generation. Cumulatively 196 participants participated in these two workshops and about 52 articles were eventually submitted for publication consideration in this monograph. The twenty-one articles in this very monograph are the articles that eventually made it through the rigorous peer-review process. We remain grateful to the reviewers for doing thorough work on the articles.

Thus, we are very pleased to present the *2018 Monograph of Atmospheric Research* which contains twenty-one articles, including some review papers, to readers in all spheres of interest across Nigeria and beyond. It is our hope that this effort will continue and will serve as a reference to atmospheric researchers in Nigeria.

**Prof. A. B. Rabi and Dr. O. E. Abiye,**  
*Editors*



## Microgravity-induced early growth and anatomical alterations *Corchorus tridens* (Bush Okra)

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### ABSTRACT

In this work, *Corchorus tridens* (bush okra) seeds were exposed to 72 hours of simulated microgravity condition using a one-axis clinostat. The seeds were sown on a 1.5% combination of plant nutrient and agar-agar solidified medium in three Petri dishes. One of the Petri dishes was mounted on the clinostat and allowed to rotate at the speed of 20 rpm for 72 hours while the others were subjected to the normal gravity vector. All are kept in a dark chamber to remove the effect of light and maintaining the same humidity and temperature. The growth rate, the roots curvature and anatomical sections of both clinorotated and normal gravity plants were made after 72 hours and observed using a digital microscope. The thickness of the epidermis and cortex of the roots of the clinorotated plant are greater than those of normal gravity. *Corchorus tridens* showed adaptive features to the microgravity stress by changing their internal cellular structure and behaviour such as elongation in the thickness of cells and increase rate of cell proliferation.

**Keywords:** anatomy, clinostat, germination, microgravity, *Corchorus tridens*

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### INTRODUCTION

Microgravity is a typical space environment, which causes an abiotic stress on living organisms. The clinostat is one of the various platforms used to simulate microgravity condition. Several researches have been conducted to investigate the responses of plants to the condition of microgravity and various altered gravity conditions (Cowls et al., 1984, Fujie et al., 1993, Hu et al., 2007, Mirsandi et al., 2015, Jing et al., 2015, Zheng et al., 2015).

The study of the modifications induced by altered gravity in functions of plant cells has been described as a valuable tool for the objective of the survival of terrestrial organisms in conditions different from those of the Earth. These changes in the gravitational environmental conditions have resulted in extensive stress for plant cells (Matia et al., 2009).

Plants grown in microgravity or simulated microgravity exhibit spontaneous auto morphogenesis (changes in growth direction) (Hoson, 2014) due to changes in plants hormones such as Auxin, Gibberellins and ethylene which serves as signal transducers responding to the changes is the gravity vector. Different plants have unique responses to the changes in gravity vector. Under microgravity conditions in space, the growth rates of many plant organs were reported to increase (Halstead and Dutcher 1987), but they were not changed or even decreased in some organs (Kiss et al. 1998, Levine et al. 2001).

In this study, the germination, early growth and anatomical responses of *Corchorus tridens* (Bush Okra) in simulated microgravity condition in the laboratory was investigated. Efforts were made to remove influence of environmental

factors such light and maintaining the same humidity in both experimental set-up.

### MATERIALS AND METHODS

Seeds of bush okra (*Corchorus tridens*): NGB 01367 used for this study were collected from The National Centre for Genetic Research and Biotechnology (NACGRAB), Ibadan, Nigeria.

The seed supporting substrate agar was prepared according to standard method (UNOOSA, 2013). 100 mL of 2.5% Duchefa Biochemie Plant Agar-Agar in tap water (2.5 g agar-agar in 100 mL of tap water) was prepared. The agar-agar was boiled and stirred until no visible particles are left (up to two minutes) i.e. a clear solution. The solution was allowed to cool down to about 60 °C. Three petri dishes were filled with 10 mL to 25 mL of the agar-agar solution. The right depth of the agar-agar solution is such that the seeds can be embedded only halfway in the agar-agar, thus guaranteeing a supply of oxygen for the seeds. The agar-agar is allowed to cool down and solidify.

The seeds of bush okra were enclosed inside a tissue paper and dipped inside a hot water for 2-3 seconds and then allowed to air dry. This was done in order to break the usual dormancy of the seeds. In each petri dish, nine seeds of the bush okra were planted on the agar-agar by using the tweezers in the same direction in order to identify the micropyle. After seeding the seeds on the agar-agar surface, two of the petri dishes were placed vertically using a petri dish holder as control and the third petri dish was mounted on the clinostat. The clinostat was rotated at a speed of 20 rpm for 72 hours (3 days) inside a growth chamber. The set-up was isolated from light using a closed chamber keeping all environmental conditions equal for both the clinorotated and the



control. The time of germination was recorded and germination percentage was calculated after germination.

After germination, one of the Petri dishes growing on normal gravity condition was rotated through 90 degrees to observe the response of the roots towards gravity vector. The pictures of the 3 Petri dishes i.e. normal gravity (1g), 90 degree rotated and clinorotated was taken at every 30 minutes using a Canon ixi 160 digital camera (20 mega pixel) for 3 hours in order to determine the growth rate and root curvature.

The root curvatures of the 90 degrees turned and clinorotated roots were determined following the standard methods (UNOOSA, 2013). It was done using an open-source image-processing application called ImageJ software. Out of the nine seedlings in each petri dish, three uniformly germinated ones were selected for measurement and analysis at each time point i.e. every 30 minutes for 3 hours. Each result represents an average of three replicates.

The growth rates were determined using standard methods (UNOOSA, 2013). Out of the nine seedlings in each petri dish, three uniformly germinated ones were selected for measurement and analysis measured using ImageJ software.

After 72 hours, free-hand fresh transverse sections of the root of the clinorotated plant and normal gravity plant in water were made using dissecting blade following the methods of Akomolafe et al., (2017) as follows: two or three drops of 1% Safranin O stain was transferred by pipette to a clean slide. The specimen was then placed by forceps to the drop of stain and left for 1 to 2 minutes. The stain was rinsed with 3 changes of distilled water. Hereafter, the stained specimens were dehydrated using ethanol. This was left for about 1 minute and rinsed with distilled water. The stained specimens were then transferred to clean slide containing a drop of dilute glycerol and covered with a clean cover slip which is placed gently at an angle to avoid air bubbles. The cover slip was sealed with transparent nail polish. The mounted specimen was hereafter placed on the digital compound microscope for microscopic observation of its anatomical features. All quantitative data were subjected to student's t-test between the normal gravity (control) and clinorotated roots for significance difference.

## RESULTS AND DISCUSSION

The seeds of the clinorotated plant started germinating earlier at 18 hours after seeding than the ones under normal gravity condition. Germination started at 42 hours for the normal gravity seeds after seeding. The percentage germination of normal gravity seeds was found to be (67%) higher than the clinorotated ones (56%). All environmental conditions were kept constant for both normal gravity and clinorotated (Table 1). The clinorotated plants did not germinate in definite pattern. The orientation of the roots was in different directions unlike the normal gravity which all germinated towards the direction of gravity vector (fig. 1).

The curvature angle of the 90 degree turned roots was higher than the clinorotated ones after 90 minutes and at 960 minutes (fig. 2) which agrees with existing results. The growth rate of the clinorotated roots and that of 1g increased with time after

germination (fig. 3). Also, at each time point after germination, the growth rate of the clinorotated was higher than 1g.

It was observed that the clinorotated roots have distinct cellular arrangement unlike those of normal gravity. The roots cells of normal gravity plants developed faster than clinorotated as the boundaries between the cells were noticeable. The root hairs of the clinorotated root were longer than the normal gravity ones. The thickness of the epidermis and cortex of the clinorotated root are higher than that of normal gravity (Table 2). Also, the number of parenchyma cells per millimeter of clinorotated plant was more than the normal gravity one.

The higher percentage germination recorded for clinorotated seeds than normal gravity ones under similar constant environmental conditions may indicate that microgravity influences the germination of seeds of *Corchorus tridens*. The orientation of the clinorotated roots which were in different directions showed that the clinorotated roots could not sense gravity in any direction. And since there was no light influence, this could mean that the clinostat was able to create a simulated microgravity condition for the roots of this plant. The 90 degree turned roots were able to sense the direction of gravity thereby leading to increase in their root curvature angle unlike those of clinorotated which were under the influence of microgravity. Also, the higher growth rate observed in the clinorotated roots than the normal gravity could be because the growth hormones responsible for early growth were enhanced in simulated microgravity. This is similar to Simonan et al., (2006) who observed fluctuations in the photosynthetic yield of some plants and attributed them to changes in gravity because series of parameters such as light intensity, temperature, pH, oxygen concentration, or obstruction of the measurements via air bubbles were kept constant. This result is contradictory to Tripathy (1996) who observed that growth rate of wheat plants grown on space stations are reduced by the microgravity environment. This could mean different plants respond differently to condition of microgravity

According to (Matia et al., 2005, 2009), seedlings grown in simulated microgravity showed a longer span than those grown in normal gravity conditions especially in the evaluation of the cell number and size in the root meristem which usually resulted in enhanced proliferation, with shortened cell size. This is similar to the distinct cellular arrangement observed in clinorotated root unlike that of normal gravity as well as the higher thickness of epidermis, cortex and number of parenchyma cells observed in the clinorotated root.

Table 1: Showing the environmental variables and growth conditions of normal gravity and clinorotated seeds

	Normal Gravity	Clinorotated
Relative Humidity at Planting	57%	57%
Relative Humidity at Germination	63%	63%
Temperature at Planting	26.1°C	26.1°C
Temperature at Germination	28.4°C	28.4°C
Percentage germination	67%	56%

Table 2. The anatomical features of the root of *Corchorus tridens*

ANATOMICAL FEATURES	CLINOROTATED	NORMAL GRAVITY
Length of root hairs (mm)	0.12 ± 0.01	0.05 ± 0.0
Thickness of epidermis (mm)	0.02 ± 0.0	0.01 ± 0.0
Thickness of the cortex (mm)	0.31 ± 0.02	0.27 ± 0.0
Diameter of vascular bundle (mm)	0.08 ± 0.01	0.13 ± 0.01
No of cell per mm	27.3 ± 2.2	17.3 ± 1.2

Value represents mean ± SE and are significantly different at  $\alpha \leq 0.05$

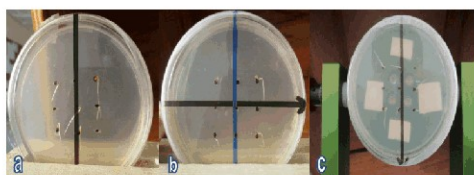


Fig. 1. (a) 1g *Corchorus tridens* (b) 90° rotated *Corchorus tridens* (c) Clinorotated *Corchorus tridens*

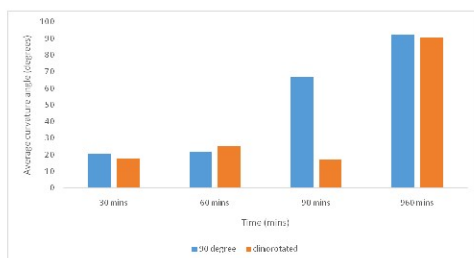


Fig. 2. Showing the average curvature angle of the clinorotated roots and 90 degree turned roots of *Corchorus tridens*

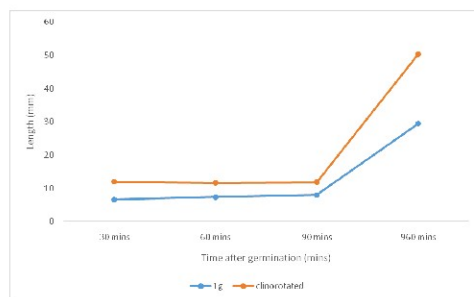


Fig. 3. Showing the effect of clinorotation on the growth rate of *Corchorus tridens*



Fig. 4. The transverse section of the clinorotated root of *Corchorus tridens*

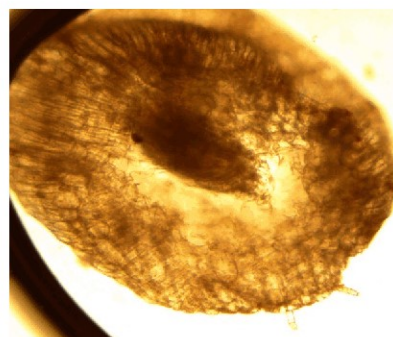


Fig. 5. The transverse section of the normal gravity root of *Corchorus tridens*

## CONCLUSION

Conclusively, these results show that microgravity influenced the germination, growth and anatomy of *Corchorus tridens*.

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**Our Mandates**

The Center for Atmospheric Research, CAR, is a research and development center of NASRDA committed to research and capacity building in the atmospheric and related sciences. CAR shall be dedicated to understanding the atmosphere—the air around us—and the interconnected processes that make up the Earth system, from the ocean floor through the ionosphere to the Sun's core. The Center for Atmospheric Research provides research facilities, and services for the atmospheric and Earth sciences community.

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