FIELD OBSERVATION OF MIRCOGRAVITY EXPOSED MAIZE SEEDS AFTER CHEMO PRIMING

BY

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(BIOCHEMISTRY TECHNIQUES)

FACULTY OF LIFE SCIENCE

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A PROJECT WORK SUBMITTED TO THE DEPARTMENT OF SCIENCE LABORATORY TECHNOLOGY, FACULTY OF LIFE SCIENCES, UNIVERSITY OF BENIN, BENIN CITY IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF BACHELORS OF SCIENCE DEGREE (B.Sc.) IN SCIENCE LABORATORY TECHNOLOGY (BIOCHEMISTRY TECHNIQUES)

MAY, 2024.

CERTIFICATION

This is to certify that this research work was carried out by Queen IMHANLAHIMI (Miss) in the Department of Science Laboratory Technology, Life Sciences, University of Benin, Benin City.

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DEDICATION

This work is dedicated to Almighty God, who in His infinite mercy gave me knowledge and composure of thoughts. And to every curious biotechnology inclined laboratory technologist, this research is also dedicated to you. May its pages expand your horizon and inspire you to seek answers to everyday questions.

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ABSTRACT

With Earth facing challenges such as increased urbanization, land degradation, pollution, and population growth, the search for alternative human settlement sites has gained momentum. Scientists are exploring space colonization as a potential solution, but microgravity presents significant obstacles to plant growth and food production in space. This study investigates the growth of maize seeds under simulated microgravity conditions using a clinostat. Maize seeds treated with growth stimulators (Vitamin C and Indole butyric acid) were subjected to clinorotation for 120 hours. Conducted at the Space-Earth Environment Research Laboratory, University of Benin, the experiment revealed morphological changes in maize seedlings exposed to microgravity. Height and length increased, while girth decreased compared to the control group. Minimal differences were observed in leaf number, sugar, and protein content between the control and microgravity-stimulated groups. These findings highlight the need for further research to understand the molecular mechanisms driving plant responses to microgravity and optimize space agriculture for future human habitation beyond Earth.

CHAPTER ONE

INTRODUCTION

1.1 Background of Study

The search for other human settlement sites has gained traction recently as the globe grows more and more congested due to increased urbanization, land degradation, pollution and population growth in all nations, regardless of their degree of scientific or economic advancement (Vandenbrink *et al*., 2014). The main concern is that the earth's carrying capacity would be exceeded, making it impossible for it to supply food, nutrients, oxygen, and other resources to the world's growing population. To prevent a possible humanitarian disaster, scientists have begun searching for alternate places to live (Braun *et al.,* 2018).

This has facilitated the study of alternate planets to determine the potential for human existence in the case of a humanitarian emergency. The success of space colonization will depend on the crew's capacity to regularly obtain oxygen, water, and food without requiring much resupply from Earth. Human exploration endeavors have frequently succeeded or failed throughout history depending on how much attention was paid to sustenance (Strohm *et al*., 2014).

Since humans are heterotrophs, resources like oxygen for the generation of metabolic energy and food would be required during the lengthy journey across space that may take years. The astronauts must be able to cycle their energy source in order to produce water, oxygen, and ready-to-eat foods including carrots, cucumbers, watermelon, maize, and cabbage. Microgravity is the main restricting factor in producing these crops in space (Kittang *et al*., 2015). Microgravity, which is commonly seen in space environments, is a state in which gravitational forces are significantly reduced. In microgravity, the force of gravity is far less than it is on Earth, giving the impression that objects and living things are in a state of perpetual free fall. This changed gravitational state presents special difficulties for many biological activities, including those essential to the growth and development of plants (Kiss *et al.,* 2019).

Research has indicated that variations in the force of gravity typically impact the growth of plants, as they encounter various difficulties when attempting to adjust to the lack of gravity. The stability of the plant overall and nutrient uptake may be jeopardized by reduced anchoring. Plants find it difficult to efficiently redistribute fluids in the absence of gravity-driven cues. When there is no gravity, signaling pathways change, which affects how the body reacts to external stimuli and may even impede adaptive mechanisms. The way cells grow and align in microgravity can have an impact on the structural stability of plant tissues. Weaker stems and a weakened overall plant architecture could arise from this. In the absence of gravity, altered fluid dynamics may have an impact on how nutrients travel through the soil. In plants, the lack of pressure gradients caused by gravity can result in an uneven distribution of water alterations in the cytoskeleton's and cell walls' orientation (Sugimoto *et al.,* 2014). Previous work done by Orukpe *et al*., 2021, showed that microgravitational effects on maize seeds significantly decreased its seed growth capacity and germinability hence the maize seeds in this work were treated with plant growth stimulators(Vitamin C and Indole butyric acid) which help in growth and developmental processes including plant height, Number of leaves per plant, growth, flowering, even fruit size resulting in increased yield and also increasing their chances of survival even in stressful situations (Ikhiajiagbe *et al.,* 2021).

For the purpose of investigating the potential of extended space travel, advancements in technology are necessary to cultivate and gather food in space. Gaining insight into how plants respond in environments with reduced or nearly negligible gravity can aid in the development of those technologies in the future.

1.2 Problem Statement

The study of how plants react to microgravity is still a continuous scientific project that aims to deepen our comprehension of basic mechanisms under the particular circumstances of space travel. While previous studies have yielded insightful information, further research is still needed to fully comprehend how maize seedlings react to microgravity. By methodically analyzing the productivity factors of microgravity-exposed maize seeds, this study seeks to advance our knowledge of plant adaptation in space and aid in the creation of cutting-edge agricultural techniques for upcoming space travel and possible extraterrestrial settlements.

1.3 Aim of Study

To ascertain how maize seeds grow in response to microgravity simulations on a clinostat, with the ultimate goal of clarifying how modified gravitational conditions affect the developmental characteristics of maize plants.

1.4 Scope of Study

I. Collection of maize grains from Uselu market Ugbowo, Benin City.

II. Grouping of sampling petri-dish

III. Treatment of samples

IV. Preparation of the agar

V. Setting up the Clinostat

VI. Monitoring germination success

VII. Analyze seedling growth patterns

VIII. Assessment of morphological changes

IX. Comparism of results with Earth-based controls

X. Correlation of field observations

CHAPTER TWO

LITERATURE REVIEW

2.1 Microgravity

Often called "weightlessness," microgravity is a special state in which gravitational forces are significantly less than on Earth. When an object or organism is in a microgravity environment, it is in a state of constant free fall, which significantly reduces the effect of gravity (Vladimir and Thais, 2020). This phenomena is a crucial component of the space environment and has a significant impact on how physical entities behave (Afolayan *et al.,* 2019). The state of considerably reduced gravitational forces, which approaches zero gravity, is called microgravity. Microgravity is the continual feeling of weightlessness that results from an object, like a spaceship or an astronaut, falling freely toward a celestial body. This is in contrast to the constant gravitational force felt on Earth.

Microgravity is the general environment outside of Earth's atmosphere in space.While orbiting celestial bodies like the Earth or other planets, spacecraft and astronauts go through this stage. In microgravity, an object's orbital velocity opposes the effects of gravity, resulting in an environment where items and living things appear to float freely inside the spaceship (Brungs *et al.,* 2016). Comprehending the subtleties of microgravity is crucial for numerous scientific fields, such as biology, agriculture, and space physiology (Oluwafemi *et al.,* 2022). Particularly when examining how living things, like plants, react and adapt to this particular environment, the changed gravitational conditions present special opportunities and problems (Bizzarri *et al.,* 2015).

2.1.1 Characteristics of Microgravity

There are distinct characteristics of microgravity that distinguish it from Earth's gravitational environment.

2.1.1.1 State of Continuous Free Fall

Things and living things descend in a constant state of free fall in microgravity, which is similar to the feeling of being weightless. As they orbit celestial planets like the Earth, this happens (Oluwafemi and Neduncheran, 2022). The sensation of weightlessness results from the object and spacecraft or celestial body falling simultaneously, not from the lack of gravity. Because of this perpetual free fall, objects appear to float within their surroundings, defying the usual limitations imposed by Earth's gravitational forces (Afolayan *et al*., 2019).

2.1.1.2 Absence of Predominant Downward Gravitational Force

The idea that there is a dominant downward gravitational force is disrupted by microgravity, in contrast to Earth, where gravity always pushes objects toward the centre. The gravitational pull is much less in microgravity situations, enabling things to achieve a state of balance between the gravitational force exerted on them and their forward velocity. As a result, objects and living things experience a state of weightlessness since there is no dominant direction of gravitational pull (Oluwafemi, 2017).

2.2 Space Agriculture

Space agriculture, also referred to as astro-agricultural or space farming, is the process of producing plants and crops outside of Earth's atmosphere (Wheeler, 2017). It includes the techniques and tools used to create food, oxygen, and other necessities for human survival in space missions and possible interplanetary settlements in a sustainable manner. By offering a consistent and sustainable supply of food and oxygen, space agriculture seeks to solve the difficulties associated with extended space voyages and the potential for human colonization of other celestial bodies in the future. It entails modifying conventional farming methods to fit the particulars of space, such as microgravity, scarce resources, and hostile environmental circumstances (Avercheva *et al.,* 2014).The ultimate goal of space agriculture is to facilitate long-term space exploration and colonization by facilitating self-sufficiency and enhancing the robustness of space habitats (Orukpe *et al.*, 2021).

2.2.1 Historical Settings and Significant Events

The advancement of space exploration has been closely linked to the continuous endeavour of developing space agriculture. Important achievements and trends include:

2.2.1.1 Early Experiments

The earliest trials pertaining to plant cultivation in space can be traced back to the initial phases of space exploration. The field of space botany was first introduced in 1960 when the Soviet Union launched Sputnik 5, a spacecraft carrying plant seeds.

2.2.1.2 NASA's Veggie Experiment

The Veggie experiment was launched in 2014 on the International Space Station (ISS) by NASA with the goal of investigating plant growth in space and its potential of supplying crew members with fresh food.

2.2.1.3 Advanced Plant Habitat (APH)

NASA's Advanced Plant Habitat (APH), which was deployed on the International Space Station (ISS) in 2017, is a noteworthy development in the field of space agriculture research. It offers regulated environmental parameters, such as humidity, temperature, and illumination, for the purpose of researching plant growth.

2.2.1.4 Bio-regenerative Life Support Systems (BLSS)

The goal of research on bio-regenerative life support systems, or BLSS, is to create closed-loop systems that incorporate plants into the recycling of nutrients, oxygen, and water. The goal of these systems is to establish self-sufficient ecosystems that can sustain both interplanetary communities and extended space journeys.

2.2.1.5 Private Sector Initiative

In recent years, private businesses have also entered the field of space agricultural research investigating novel strategies for producing food in space and helping to bring space-based agriculture technologies to market.

2.2.2 Challenges in Space Agriculture

2.2.2.1 Microgravity

Basic processes including nutrient intake, water distribution, and plant cell growth are altered by the presence or absence of microgravity (Massa *et al.,* 2016).

2.2.2.2 Limited Resources

Water, soil, and energy are among the few resources found in space habitats. Good resource management is essential to long-term plant growth.

2.2.2.3 Harsh Environment and Conditions

Micrometeoroids, radiation exposure, and extremely high temperatures all have an impact on plant development and health in space conditions (Monje *et al*., 2020).

2.2.2.4. Closed Ecosystems

Plants in closed-loop life support systems must effectively recycle nutrients, water, and air, which makes it difficult to keep the environment steady (Kiss *et al*., 2019).

2.2.2.5 Space Restrictions

Because there isn't much room in spacecraft and housing for plant cultivation, small and effective growing methods must be created.

2.2.3 Objectives of Space Agriculture

2.2.3.1 Food Production

The main goal of space agriculture is to give astronauts on extended space journeys and possible interplanetary settlements a reliable supply of fresh food. This involves cultivating an array of nutrient-dense crops to uphold a well-rounded diet.

2.2.3.2 Life Support

By generating oxygen through photosynthesis and eliminating carbon dioxide from the atmosphere, plants are essential components of closed-loop life support systems. Optimizing these processes to sustain human life in space habitats is the goal of space agriculture.

2.2.3.3 Resource Utilization

In order to increase crop yields and reduce waste, space agriculture aims to make effective use of scarce resources, including water, nutrients, and energy.

2.2.3.4 Psychological Well-Being

Growing plants in space habitats helps astronauts feel less stressed, happier, and more connected to the natural world. The goal of space agriculture is to improve crew member's mental health over extended deployments.

2.2.3.5 Research and Innovation

Research on space agriculture stimulates advances in plant biology, engineering, and technology, advancing both space exploration and terrestrial agriculture.

2.3 Role of Plants in Space Missions

2.3.1 Oxygen Production

Photosynthesis is the process by which plants use sunlight to change carbon dioxide into oxygen. During extended expeditions this feature is essential for replenishing the oxygen supply in the cramped spacecraft.

2.3.2 Removal of Carbon Dioxide

Plants absorb carbon dioxide during photosynthesis, which aids in controlling and preserving the ideal concentrations of this gas inside the spaceship. Preventing the build-up of surplus carbon dioxide is crucial as it may pose a threat to astronauts.

2.3.3 Closed-Loop Life Support Systems

Modelled after Earth's natural ecosystems, closed-loop life support systems rely heavily on plants. In these systems, plants help recycle nutrients, water, and air, resulting in a self-sufficient ecosystem that is ideal for long-term space journeys (Raymond, 2017).

2.3.4 Psychological Well-Being of Astronauts

Having greenery and living plants nearby can have a good effect on astronauts' psychological health. During prolonged confinement, interacting with plants fosters a sense of connection to nature, reduces stress, and improves mental health.

2.4 Microgravity's Impact on plant physiology

2.4.1 Nutrient Uptake Under Microgravity Conditions

Plants' typical systems of nutrient uptake are greatly modified in microgravity, which affects how well they are able to absorb vital nutrients from the soil. These modifications are a result of two major factors:

2.4.1.1 Diminished Gravitational Guidance

Roots use gravitropism, a directed growth response, to direct their search for nutrients in the soil when gravity is present on Earth. But in microgravity, this process is upset by the lack of reliable gravitational cues, leading roots to grow erratically or even lose orientation (Farooq *et al.,* 2024). This can make it difficult for roots to get nutrients that are dispersed unevenly throughout the substrate and prevent them from penetrating the soil effectively.

2.4.1.2 Altered Capillary Action

Water and dissolved nutrients are more easily transported into the roots by capillary action, which is somewhat dependent on gravity. In microgravity, capillary action is impacted by the lack of gravity-induced pressure differences, which may interfere with plant roots' typical intake of water and nutrients. Water and nutrients might not be distributed equally within the plant without the gravitational pull to help with fluid mobility. This could cause imbalances in nutrient uptake and possibly jeopardize the health of the plant (Hoson, 2014).

2.4.2 Water Distribution in Microgravity

Plant's ability to absorb and transport water is significantly impacted by the considerable changes in water distribution that occur in microgravity circumstances. These modifications, which mostly originate from the lack of gravitational pull, affect vital functions like transpiration, capillary action, and the behaviour of water itself (Kiss, 2014).

2.4.2.1 Changes in Transpiration

Gravitational forces have an impact on transpiration, which is the process by which water is taken up by plant roots and then released into the atmosphere through stomata in the leaves (Croce *et al.,* 202). The absence of gravitational force in microgravity reduces the downward flow of water in the vascular system of the plant, which may change transpiration rates. Changes in transpiration rates can cause the plant's water distribution patterns to become uneven, which can have an impact on nutrient transport and hydration levels(Vandenbrink *et al.*, 2014).

2.4.2.2 Disruption of Capillary Action

Capillary action, which relies on the cohesive and adhesive properties of water and the presence of gravity, facilitates the movement of water and nutrients through plant tissues (Hoson and Wakabayashi, 2015). In microgravity, the absence of gravitational forces disrupts the dynamics of capillary action, potentially impeding the upward movement of water from the roots to the shoots. This disruption may hinder the efficient distribution of water and nutrients within the plant, affecting overall hydration and nutrient uptake (Medina *et al.,* 2021).

2.4.2.3 Formation of Fluid Spheres

Because there are no pressure gradients brought on by gravity in microgravity, water behaves differently. Instead of sticking to surfaces like it does on Earth, water forms spherical formations called fluid spheres. The way water interacts with plant surfaces may change as a result of the development of fluid spheres, which could have an impact on the plant's distribution and processes for absorbing water (Kiss, 2019).

2.4.3 Cell Growth and Development under Microgravity Conditions

2.4.3.1 Effects of Microgravity on Cell Division

The absence of gravitational cues in microgravity situations throws off the cell cycle's regular control (Zheng *et al*., 2015). The intricate sequence of events that make up the cell cycle may be difficult for cells to coordinate without the spatial orientation that gravity provides. Microgravity can cause changes in the rates at which cells divide, leading to either more or less proliferation based on the particular circumstances and types of cells involved (Mosa *et al.,* 2017). Non-uniform patterns of cell proliferation within tissues can also result from anomalies in the timing and spatial orientation of cell division events caused by the absence of gravity cues. Studies have shown that, in comparison to Earth-based controls, cell growth rates rise under microgravity settings (Böhmer and Schleiff, 2019).

2.4.3.2 Impact on Structural Integrity and Morphology

The lack of gravity-related mechanical cues in microgravity causes abnormal cell wall component deposition and arrangement (Rioux *et al.,* 2015). Plant cell's structural integrity may be jeopardized by this modification, which may result in abnormalities in the structure of the cell walls. When compared to plant cells grown under normal gravitational conditions, those grown in microgravity may have asymmetrical sizes and forms. Cell shapes and sizes can become twisted or aberrant when there are no gravitational cues for cell orientation and growth (Kordyum, 2014).

2.5 Maize (Zea mays)

Zea mays ssp, the corn plant, is native to Mexico and Central America. It is a member of the Poacee family and tribe Maydae. Its genome is 2.3 gigabases in size, with 20 somatic chromosomes, and about 32,000 genes (Hossain *et al.,* 2016). The versatility of maize allows it to thrive in a wide range of agroecologies and sets it apart from other crops. Because it can be used for both human and animal nutrition and as a vital component of many different industrial products, it has become a crop of worldwide significance. In addition, maize is used globally as a model organism in scientific research. Of the primary staple cereals, maize is produced worldwide at the highest rate, 1016.73 million metric tonnes annually (FAOSTAT 2013).

Plate 1: Orange yellow and white variety of maize

Source: (Chautara, 2016).

Since maize is an essential source of calories and proteins for billions of people living in developing nations, especially in Africa, Mesoamerica, and Asia, a large percentage of the maize produced worldwide is utilized for animal consumption. Moreover, it provides vital vitamins and minerals that the human body needs. For about 4.5 billion people in 94 developing nations, maize accounts for at least 30% of total calories, along with rice and wheat. With a population of over 310 million, maize accounts for more than 20% of calories in human diets in 21 nations and more than 30% in 12 countries. Though projections show that by 2050, the developed world will use more maize than the developing world does now (Prasanna, 2014). In space research, maize presents special benefits for long-term human settlement and space travel. Because of its adaptability to a wide range of climatic circumstances, it is a good choice for growing in controlled settings like space habitats or outposts on the moon and Mars (Chaudhary *et al.,* 2014). Furthermore, maize is a good option for producing fresh food and oxygen in closed-loop life support systems due to its quick growth rate and high biomass output (Chavan and Smith, 2014). Although studies as the ones carried out by Orukpe *et al.,* have demonstrated that maize seeds can sprout and develop in microgravity settings, more investigation is required to comprehend the ways in which space circumstances affect the physiology and growth patterns of maize. These studies are critical to improving farming methods and creating hardy crop types that may endure the harsh conditions of spaceflight, such as radiation, microgravity, and scarce supplies. Furthermore, maize has potential applications in bio-regenerative life support systems in space, where plants are essential for recycling nutrients, water, and air. Closed-loop ecosystems in space habitats are more sustainable because of maize's ability to produce oxygen and efficiently absorb and use carbon dioxide through photosynthesis.

2.5.1 Maize Seeds in Microgravity (key findings from previous experiments)

2.5.1.1 Germination and Seedling Growth

The ability of maize seeds to germinate and start seedling growth under microgravity settings emphasizes the crop's adaptability to changing gravitational surroundings. When compared to seedlings produced in Earth-based environments, notable variations in growth patterns have been observed. These differences highlight how microgravity affects maize seedling development in its early stages (Zabel *et al.,* 2016). Compared to their terrestrial counterparts, maize seedlings in microgravity have different growth rates, morphologies, and general developmental patterns. These variations show up as variations in leaf shape, shoot development, root elongation, and general seedling vigour (Ikhajiagbe *et al.,* 2022). Microgravity conditions cause disruptions to normal growth processes due to the lack of gravitational cues, which in turn causes differences in the physiological responses and seedling architecture.

2.5.1.2 Root Growth

Studies on maize seedlings grown in microgravity environments have shown notable changes in root development patterns. These alterations include differences in root length, branching, and orientation, suggesting that the development and architecture of maize roots are significantly impacted by the lack of gravity. Maize seedlings in microgravity conditions show variations in root elongation, including changes in the total length of the main roots and the development of lateral roots (Musa and Ikhajiagbe, 2021). Furthermore, changes in the density and distribution of root branches may result from disruptions to the branching pattern of maize roots. Moreover, the direction of root growth is not in line with the usual downward orientation seen on Earth since the gravitational cue that directs root tropisms is absent (Oluwafemi and Olubiyi, 2019).

2.5.1.3 Shoot Growth

Maize seedlings grown in microgravity conditions exhibit changes in many aspects of shoot growth, indicating the impact of microgravity on plant structures above ground. These adaptations include variations in the length of the stem, modifications to the morphology of the leaves, and adjustments to the amount of chlorophyll (Oluwafemi, 2021).

2.5.1.3.1 Stem Elongation

Compared to seedlings produced in normal gravitational settings, maize seedlings cultivated in microgravity conditions show more stem elongation (Mshelbula *et al.,* 2015). The lack of mechanical cues associated with gravity cause seedlings to grow longer stems as they look for stability and direction in the microgravity environment.

2.5.1.3.2 Modifications to Leaf Morphology

Microgravity have an impact on the size, shape, and texture of maize seedlings' leaves. These alterations result from perturbations to the regular processes of growth that are controlled by gravity, which causes differences in the patterns of leaf development (Orukpe *et al.,* 2021).

2.5.1.3.3 Alterations in Chlorophyll Content

The amount of chlorophyll, which is necessary for photosynthesis and overall plant health, may be impacted by microgravity circumstances. Studies have shown that maize seedlings grown in microgravity environments differ in terms of chlorophyll content from those grown on Earth. Plant growth and yield are impacted by these modifications because they affect photosynthetic efficiency (Koryum and Chapman, 2017).

2.5.1.4 Reproductive Development

The investigation of maize plant’s reproductive development in microgravity settings has received less attention. Although some research has suggested possible impacts on pollination, seed set, and floral growth, there are still few thorough studies in this field.

2.6 Clinostat as a Simulation Tool

Plate 2: A 2-Dimensional Clinostat

Photo credit: (Imhanlahimi, 2024).

2.6.1 Principle

A clinostat is a laboratory device used in laboratories that rotates samples constantly to mimic microgravity conditions and negate the macroscopic effects of gravity. Clinostats allow for the study of biological reactions in simulated microgravity by introducing a gravitational force-free environment through the continual rotation of biological samples (Hasenstein and Karl, 2022)

2.6.2 Functionality

Clinostats offer a regulated laboratory setting where biological specimens, such plants or cells, undergo a regular rotation. Because of the constant rotation, gravitational forces act uniformly in all directions, creating a situation similar to weightlessness or free fall. Clinostats allow scientists to isolate and study the particular impacts of simulated microgravity on biological processes by removing the gravitational force.

2.6.3 Applications

Many scientific disciplines use clinostats to study how simulated microgravity affects a variety of biological processes. Studies on plant growth, tissue development, cell differentiation, and physiological responses are some of these uses. Through simulating microgravity and subjecting biological samples to it, scientists can learn more about how organisms respond and adapt to changes in their gravitational environments.

2.6.4 Advantages

Accessible and Affordable: Clinostats offer a more affordable and accessible way to

investigate microgravity reactions than spaceflight missions. They don't require any particular infrastructure or equipment to be easily installed and operated in laboratory settings.

Controlled Experiments: Clinostats provide exact control over the characteristics of the environment, the rotation speed, and the orientation of the sample. This makes it possible for scientists to explore the effects of particular factors on biological systems through controlled experiments and variable manipulation.

Analysis of Gravitational Effects: Clinostats allow scientists to identify and analyze the particular impacts of microgravity circumstances. gravity on biological systems by reproducing

2.7 Other Expermental Approaches in Microgravity Research

2.7.1 Parabolic Flight Studies

Principle: Parabolic flight maneuvers involve aircraft flying in a parabolic trajectory to produce short periods of weightlessness.

Functionality: During parabolic flights, researchers and experiments experience brief periods of microgravity, enabling the study of biological responses to altered gravitational conditions.

Applications: Parabolic flight studies are used to investigate the immediate effects of microgravity on biological samples, including changes in gene expression, protein synthesis, and physiological responses.

Limitations: Parabolic flight experiments have short durations of microgravity, typically lasting for only 20-30 seconds per parabola. This restricts the scope and duration of experiments compared to space-based research.

2.7.2 Space-based Experiments (Experiments on the international Space Station)

Source: (Pletser and Russomano, 2020).

Plate 3: The International Space Station (ISS) is the first major international project that includes 14 countries in its realization: The USA, Russia, Canada, Japan and 10 European countries (France, Germany, Italy, Belgium, the Netherlands, Spain, Sweden, Switzerland, Denmark and Norway). With a total mass of 440 tons (but weighing 0 kg), the ISS is in low earth orbit between 400 and 450km altitude at 51.6° inclination. Since November 2000, the station is inhabited by permanent international crews (photo: NASA).

Principle: Conducting experiments in space environments, such as those aboard the International Space Station (ISS), provides extended exposure to true microgravity conditions.

Functionality: Space-based experiments allow for the study of biological responses to long-term microgravity exposure, offering insights into adaptive mechanisms and physiological changes in plants and other organisms.

Applications: Space-based research enables the investigation of complex biological processes, such as plant growth and development, in the absence of gravitational cues. It facilitates the validation of ground-based findings and the exploration of long-term effects on biological systems.

Challenges: Space-based experiments require extensive planning, resources, and collaboration with space agencies. Access to space platforms like the ISS is limited and subject to scheduling constraints, making it challenging to conduct experiments on short notice.

CHAPTER THREE

3.1 Site Location

The experiment was carried out at the Space-Earth Environment Research Laboratory, University of Benin operated by Centre for Atmospheric Research of the National Space Research and Development Agency (NASRDA). The research was carried out from January to April of 2024.

3.2 Materials

3.2.1 Collection of Sample (Maize Seeds)

Maize seeds was obtained from Uselu Market, Ovia, North-east, Ugbowo, Benin City.

3.2.2 Equipments/Laboratory Apparatus Used

1. 2-D clinostat

2. Digital weighing balance

3. Hot plate

4. Peal tape

5. Hand gloves/ Laboratory coats

6. Plastic can

7. Petri dish

8. Beaker

9. Stirring rod

10. Thermometer

11. Ruler

12. Tweezer

13. Ink marker

14. Filter paper

15. Hand trowel

3.2.3 Reagents/Chemicals Used

1. Distilled water

2. Agar

3. Plant stimulants: Vitamin C 500ppm and Indole butyric acid 500ppm

3.3 Treatment of seeds

The maize seeds were primed with water and also with selected growth stimulators before they were sowed and exposed to clinorotation for 120hrs. The seeds were pretreated with 500ppm Vitamin C and 500ppm Indole-3-Butyric Acid. Seeds were soaked in the above treatment for 60 minutes.

Plate 4: Chemo priming with vitamin C before sowing

3.4 Preparation of Agar

Preparation followed standard methods as used by Orukpe *et al,* (2021). The agar was prepared by weighing 1g in 70ml of distilled water. The solution was then heated for 10 minutes with continuous stirring to achieve a homogeneous solution. After boiling and a clear solution was observed, it was allowed to cool for about 10 minutes after pouring it on the petri dishes label g and ug, sowing followed immediately.

3.5 Sowing

Sowing was done after the agar was observed to be cool, by inoculating the petri dishes with maize seeds, with the seeds lying sideways. Two petri dishes were inoculated at each batch interval with 20seeds contained in each Petri dish. It was allowed to stay 30 mins after sowing and then sealed off properly. The petri dish containing the control was left under the influence of gravity on a table, while the seeds meant for microgravity stimulation were placed on the clinostat for 120 hours under 0.5 rpm rotation.

Plate 5: The arrangement of seeds in petri dish before placement onto the Clinostat

3.6 Clinorotation

The experiment was prepared in three batches. The first batch were placed in clinoratation at 0.5pm and studied for 120 hours, while the second batch were first exposed to growth stimulation in Vitamin C and the third batch exposed to growth stimulation In indole-3-butyric acid, before subjecting them to clinorotation at 0.05rpm in the 2-D Clinostat (Dimension clinostat,UN. New York, USA) and also studied for 120hrs for each batches. In the both batches, the control seeds were placed on the laboratory bench.

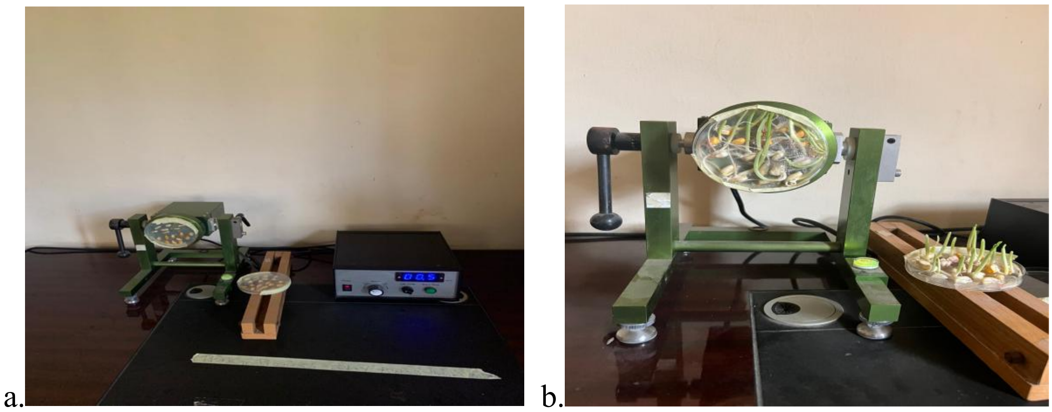

Plate 6: Petri dish mounted on the Clinostat with the control group vertically placed on the stand.

a. Petri dish immediately after sowing

b. Petri dish 120hrs after Clinorotation

3.7 Transfer to Nursery

For each batch, plastic bottles were selected and cut in halves to create containers for the experiment. The bottles were divided into two groups: one designated for microgravity conditions and the other for gravity conditions. Each bottle was filled with loamy soil and water was added to achieve optimal moisture levels. The filled bottles were allowed to cool for one hour to stabilize the soil temperature. Following the cooling period, the petri dishes containing the sprouted maize seedlings were removed from the clinostat. The seedlings were carefully removed from the petri-dish and planted in the prepared plastic bottles. Two maize seeds were planted in each bottle to ensure robust growth and facilitate comparison between samples.

The planted bottles were then transferred to a nursery environment where they were maintained for a period of 120 hours. During this time, the seedlings were provided with appropriate light, temperature, and moisture conditions to support healthy growth and development. After the 120-hour period elapsed, the seedlings were ready for transplantation to the field for further experimentation and observation.

Plate 7: The maize seedlings in Nursery

3.8 Transplanting from Nursery to Field

The process of transplanting the maize seedlings from the nursery to the field involved several steps to ensure proper establishment and growth. Each batch of seedlings was transplanted at different intervals, with a 120-hour interval between each batch to facilitate staggered planting and data collection.

To prepare the field for transplantation, ridges were formed to create raised beds for the seedlings. Grasses were added to the ridges to provide additional support and protection for the young plants. The ridges were then covered with sand using the mulching method to conserve moisture and create a favorable environment for root growth. Before transplanting, water was poured into the ridges to moisten the soil, ensuring optimal conditions for seedling establishment. The temperature at the time of planting was at 37 degrees Celsius. Transplanting commenced by carefully opening each plastic bottle and gently transferring the contents, including the seedlings and soil, into the prepared ridges. This process was repeated for all containers, ensuring uniformity in planting across the experimental batches. Upon completion of transplanting, water was evenly distributed over the planted seedlings to provide immediate hydration and promote root establishment. Additionally, special care was taken to separate the microgravity plants from the gravity plants to maintain experimental integrity and facilitate accurate data collection throughout the growing period

Plate 8: Maize seedlings transferred from nursery to Field

3.9 Growth stages of the Maize plant

a.

b.

c

Plate 9: Young maize plants

a. One week after planting

b. Two weeks after planting

c. Three weeks after planting

Plate 10: Tassel formation/Flowering

plate 11: Silk/Ear/Fruit formation

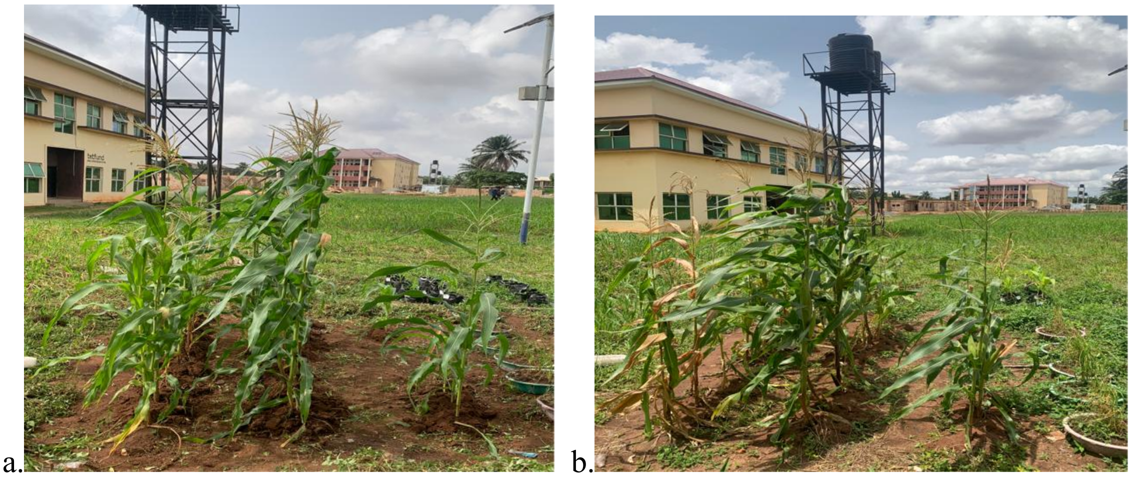

Plate 12: Matured maize plants

a. Thirteen weeks after planting

b. Senescence

CHAPTER FOUR

RESULTS

a.

b.

c.

Plate 9: Morphological parameters measured

a. The experimental work design

b. Height and number of leaves measured

c. Girth measured

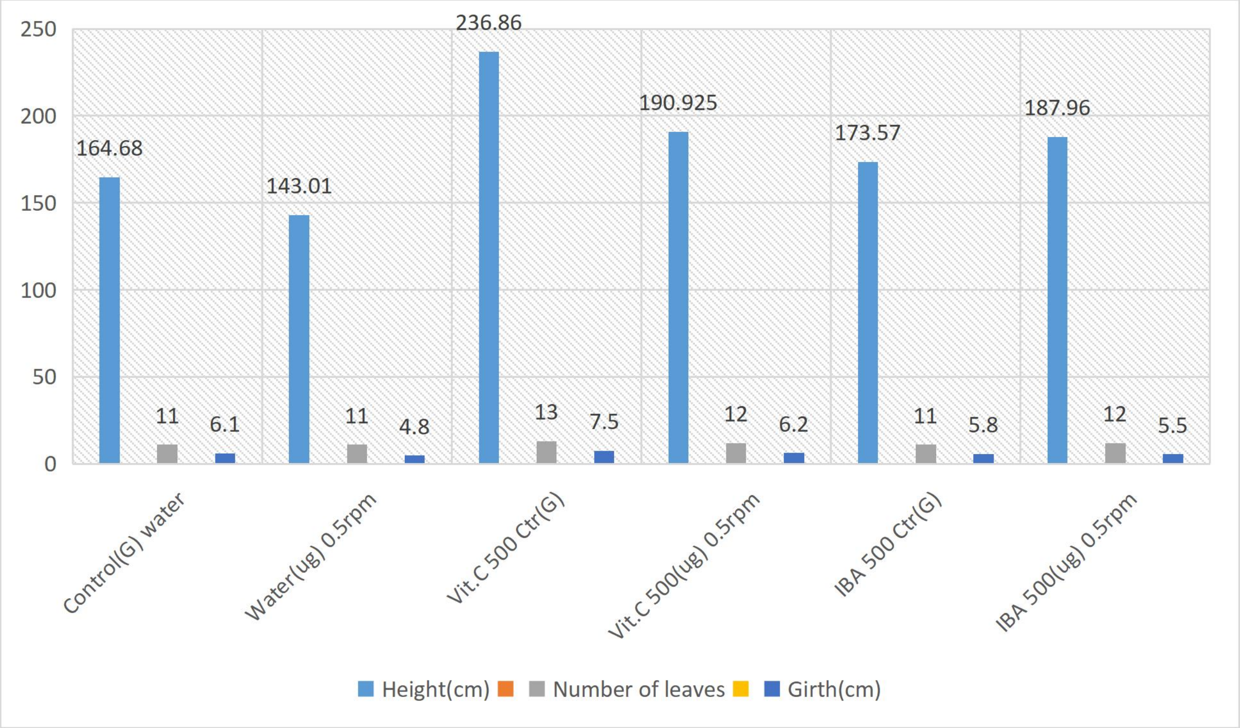

Figure 1: Morphological parameters

Keys: Vit.C- Vitamin C, IBA- Indole butyric acid, Ctr- Control, G- Gravity

Plate 10: Samples of the Maize curbs

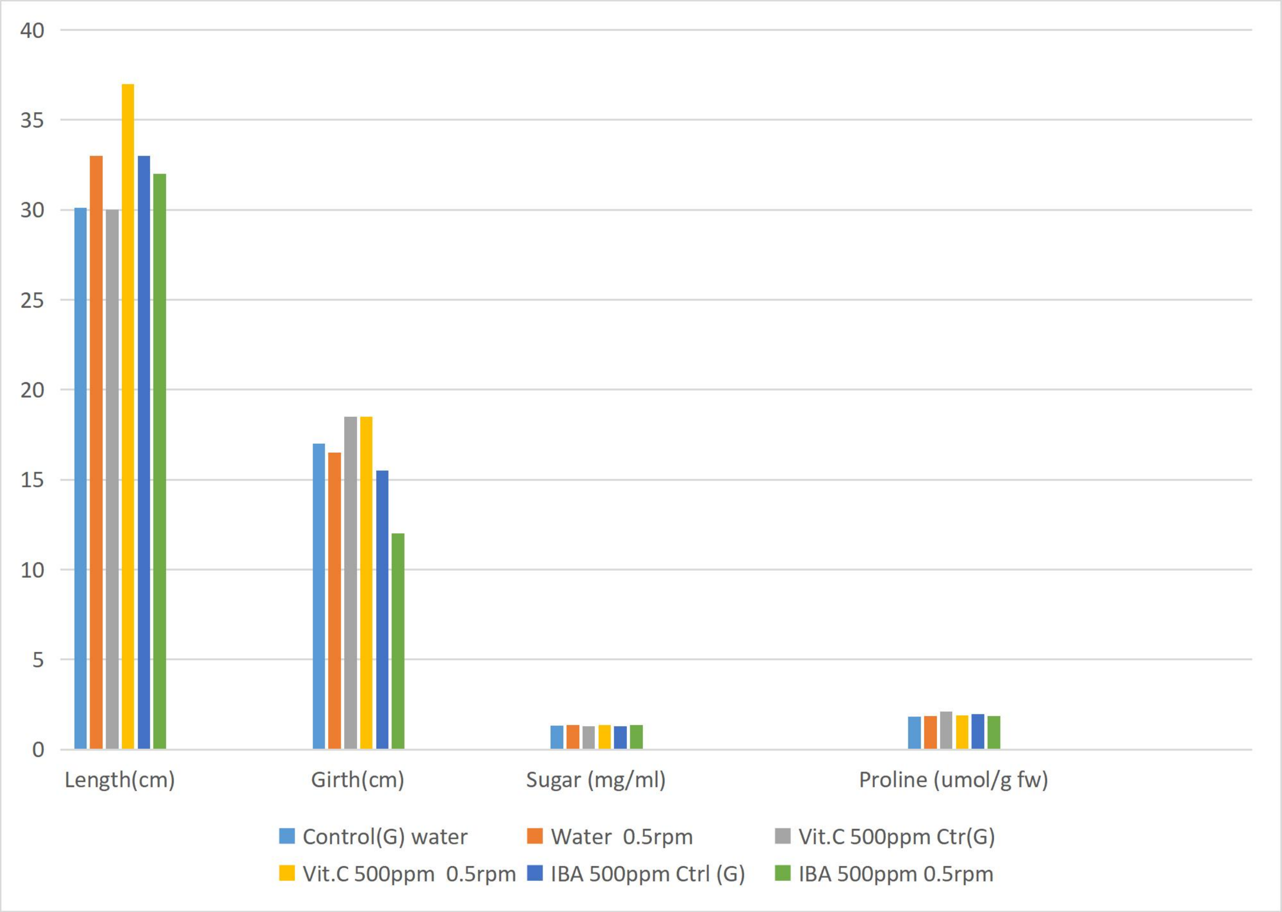

Figure 2: Curb Measurements

Keys: Vit.C- Vitamin C, IBA- Indole butyric acid, Ctr- Control, G- Gravity

CHAPTER FIVE

DISCUSSION

In this study, the aim was to carry out field observations on maize seedlings exposed to microgravity at 0.5rpm(revolution per minute) after chemo priming with Vitamin C and IBA(Indole butyric acid) in 500ppm concentrations.

The data from the field observations indicate that microgravity stimulation has a notable positive effect on both the height and length of maize plants when compared to the control group. For instance, the comparison between the control group (G) water treatment and the microgravity-stimulated treatment at 0.5 rpm; the control group had an average height of approximately 143.68 cm,while the microgravity-stimulated treatment at 0.5 rpm resulted in an increased average height of approximately 164.01 cm. Similarly, for length measurements; the control group had an average length of approximately 30.1 cm, while the Microgravity-stimulated treatment at 0.5 rpm showed an increased average length of approximately 33 cm. These findings are consistent with the known responses of plants to microgravity conditions according to previous works done by Orukpe *et al.*, 2021.

In the absence of gravitational force or under altered gravitational conditions, plants often exhibit elongated growth patterns. This phenomenon is a result of plants adapting to the altered environmental cues, particularly gravity. Without the typical downward pull of gravity, plants may prioritize vertical growth, leading to taller and longer structures.(Ikhajiagbe *et al.*, 2021)

Furthermore, the observed elongation in both height and length suggests that microgravity stimulation may influence cell elongation processes, hormonal regulation, or cell division patterns in maize plants as these mechanisms are what contribute to the overall growth response and structural changes observed in the microgravity-stimulated plants.

Regarding the girth of maize plants under microgravity stimulation compared to the control group. While microgravity stimulation had a positive impact on height and length, it resulted in a decrease in girth compared to the control group which had an average girth of approximately 6.1 cm with microgravity-stimulated treatment at 0.5 rpm showing a decreased average girth of approximately 4.8 cm.

This decrease in girth suggests that the maize plants under microgravity conditions may exhibit a different growth pattern or structural response compared to plants in normal gravity conditions. While the vertical growth is enhanced, indicating elongation, there may be alterations in lateral growth or overall plant architecture.

Several factors could contribute to this girth reduction under microgravity stimulation. It could be related to changes in cell division rates, cell expansion processes, or the distribution of growth-promoting hormones within the plant. Alterations in gene expression and signaling pathways involved in growth regulation may also play a role.(Olowoake, 2017).

No significant difference was seen in the number of leaves between the control group and the microgravity-stimulated group. This comparison suggests that microgravity stimulation does not have a pronounced effect on leaf initiation or development in maize plants within the observed time frame. As both the control group and microgravity- stimulated treatment at 0.5rpm had an average of 11 leaves per plant. This consistency in the number of leaves between the control and microgravity-stimulated groups indicates that leaf initiation and development processes in maize plants may not be significantly influenced by microgravity conditions during the observed time period.

However, it's important to note that longer-term studies or analysis at different developmental stages may reveal subtler effects on leaf growth and morphology. Microgravity-induced changes in hormonal signaling, nutrient uptake, or stress responses may manifest gradually and become more apparent over extended periods of growth and development.

The differences in sugar and protein content between the control group and the microgravity-stimulated group are minimal. This observation suggests that microgravity stimulation may not have a substantial impact on these biochemical parameters in matured maize plants within the observed time frame. The control (G) water treatment had an average sugar content of approximately 1.332 mg/ml and an average protein content of approximately 1.825 umol/g fw, while microgravity-stimulated treatment at 0.5 rpm showed similar average sugar content of approximately 1.377 mg/ml and a slightly lower average protein content of approximately 1.875 umol/g fw.

The minimal differences observed in sugar and protein content between the control and microgravity-stimulated groups suggest that microgravity stimulation may not induce significant alterations in these biochemical parameters at the observed maturity stage of maize plants. However, it's essential to consider that biochemical responses to microgravity can vary depending on various factors such as plant species, growth stage, duration of exposure, and environmental conditions.

With the data collected, it’s can be seen that microgravity stimulation can induce significant changes in certain morphological parameters such as height and length, while having minimal effects on other parameters like girth, leaf number, sugar content, and protein content. Further investigations, particularly focusing on underlying molecular mechanisms and long-term effects, would provide deeper insights into how maize plants respond to microgravity conditions and adapt their growth and biochemical processes accordingly.

CONCLUSION

This experiment has provided valuable insights into the effects of microgravity exposure on maize seedlings, how they respond to microgravity conditions and adapt their growth patterns and biochemical processes, showcasing significant morphological changes such as increased height and length, along with minimal alterations in girth, leaf number, sugar content, and protein content. These findings contribute to our understanding of how plants respond to altered gravitational conditions and have implications for future research in space agriculture and plant adaptation to extreme environments, and the optimization of growth conditions for crops under altered gravitational forces.

Further research is recommended to delve deeper into the molecular and physiological mechanisms underlying the observed changes, especially regarding girth reduction and biochemical responses. Long-term studies and analysis at different developmental stages could reveal subtler effects and provide a more comprehensive understanding of the impact of microgravity on maize plant growth and physiology.

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APPENDIX

Table 1: Results for Morphological Parameters

Morphological Parameters of the Matured Maize Plants

Sample ID	Height(cm)	No. of leaves	Girth(cm)
Control(G) water	143.68	11	6.1
Water 0.5rpm	164.01	11	4.8
Vit.C 500 Ctr(G)	190.86	13	7.5
Vit.C 500ppm 0.5rpm	236.925	12	6.2
IBA 500 Ctr(G)	173.57	11	5.8
IBA 500ppm 0.5rpm	187.96	12	5.5

Table 2: Results for Curb Measurements

Data for the curb measurements

Sample ID	Length(cm)	Girth(cm)	Sugar (mg/ml)	Protein (umol/g fw)
Control(G) water	30.1	17	1.332	1.825
Water 0.5rpm	33	16.5	1.377	1.875
Vit.C 500ppm Ctr(G)	30	18.5	1.291	2.112
Vit.C 500ppm 0.5rpm	37	18.5	1.356	1.891
IBA 500ppm Ctrl (G)	33	15.5	1.283	1.967
IBA 500ppm 0.5rpm	32	12	1.345	1.871