DETERMINATION OF THE GERMINATION PARAMETERS OF MAIZE SEEDS SUBJECTED TO CLINOROTATION

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A PROJECT WORK SUBMITTED TO THE DEPARTMENT OF SCIENCE LABORATORY TECHNOLOGY, FACULTY OF LIFE SCIENCES, UNIVERSITY OF BENIN, BENIN CITY

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CERTIFICATION

This is to certify that this project work was carried out by Faith Omokheme YUSUF (MISS) with matriculation number LSC1706157 of the Department of Science Laboratory Technology, Faculty of Life Science, University of Benin, Benin City, Edo State.

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I, YUSUF OMOKHEME FAITH, hereby declare that this research work was carried out by me in the Department of Science Laboratory Technology, Faculty of Life Science, University of Benin, Benin City.

______________________ ___________________

SIGNATURE DATE

DEDICATION

This project work is dedicated to God Almighty for his unending love and for knowledge and understanding, protection and preservation.

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I am grateful first of all to my parents Mr. and Mrs. Yusuf for all their love and support throughout the period of my study in the university of Benin, it wasn’t easy for u both but thank God.

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I would also love to appreciate my friends, Tombri Favour, Akpoka phoebe, Uleh Sylvia, ukpebor Honora and all the members of biotechnology option for all your support and encouragement over time.

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ABSTRACT

Over the years, space exploration has witnessed so many challenges especially in area of life support system which has hindered manned space craft for long space exploration. One of the major limiting factors in space life support systems is gravitational force. Plants are a vital component of life support systems in space exploration because they provide essential components for humans' long-term extraterrestrial survival. They can be used in bio-regenerative life support systems (BLSS) as sources of food and oxygen, for the absorption of CO2, and for the recycling of waste during space missions, they can also improve the atmosphere in enclosed spaces, as well as lowering the risk of mental health for astronauts. Clinorotation, a technique that simulates microgravity conditions, has gained significant interest in plant research due to its potential implications for space agriculture and understanding terrestrial plant growth responses and biological life support systems. The design involved subjecting maize seeds to clinorotation conditions within a controlled laboratory setup. The experiment was conducted in multiple replicates, with conventional germination conditions serving as the control group. The clinorotation apparatus, designed to simulate microgravity conditions, facilitated continuous rotation of the maize seeds, removing the effects of gravitational forces. Germination parameters, including germination percentage, germination rate, mean germination time, and other associated growth metrics, were meticulously measured and analyzed. Additionally, various physiological parameters, such as root and shoot lengths, were assessed to provide a comprehensive evaluation of the maize seed's germination under clinorotation stress. The results demonstrated significant variations in germination parameters between the clinorotation-exposed and control groups. The clinorotated maize seeds exhibited faster germination initiation, increased germination percentage, and altered germination rates compared to their terrestrial counterparts. The findings from this study provide crucial insights into the effects of microgravity simulation on maize germination and early growth stages. Understanding these responses is essential for the successful cultivation of crops during prolonged space missions and the establishment of extraterrestrial habitats.

CHAPTER ONE

INTRODUCTION

Microgravity is the condition whereby people or objects appear to be weightless and experience no force of gravity. It is sometimes referred to as “zero gravity” But Micro means "very small", therefore this means that gravity is not totally absent but present in very small amount. The effects of microgravity can be observed when astronauts and objects are suspended in space. Microgravity can be experienced in other ways as well. For example, you can easily move heavy objects, astronauts can move devices weighing hundreds of pounds with their fingertips (NASA, 2017). In the quest for space exploration and future human colonization of other celestial bodies, understanding the effects of microgravity on biological processes becomes crucial. One area of particular interest is how plants, essential for sustaining life, respond to altered gravitational conditions. The phenomenon of clinorotation, which simulates microgravity conditions provides a unique opportunity to investigate the impact of reduced gravity on plant germination (Paul *et al.,* 2019)

The successful cultivation of crops in space or on other celestial bodies is pivotal for sustainable human exploration and colonization beyond earth. Space missions demand self-sufficiency in food production, as transporting supplies from earth would be costly and impractical for extended missions. Understanding the responses of maize seeds to clinorotation will enable scientists and future astronauts to optimize plant growth conditions in space habitats, ensuring reliable food supplies and advancing space exploration goals (Vandenbrink *et al*., 2016)

Maize (*Zea mays*), as a widely cultivated and nutritionally important crop, serves as an ideal candidate for studying the effects of clinorotation on germination parameters. Germination is a pivotal stage in the plant's life cycle, as it marks the transition from a dormant seed to a developing seedling. It involves various physiological and biochemical processes that may be influenced by altered gravitational forces. Understanding how maize germination responds to clinorotation will shed light on the fundamental biology of plant growth under altered gravity, providing essential knowledge for space agriculture and controlled environment farming beyond Earth (Paul *et al.,* 2019).

This research project will involve a series of controlled experiments using clinostat devices that can simulate microgravity environments. The germination of maize seeds will be observed and analyzed, with a focus on key parameters such as germination percentage, germination rate, root length, shoot length, and other relevant morphological and physiological characteristics.

1.1. AIM

The aim of this project is to determine the germination parameters of maize subjected to clinorotation, providing valuable insights into how microgravity conditions affect early plant development.

1.2. SPECIFIC OBJECTIVES

The primary objectives of this research project are as follows:

1. To subject maize seeds to clinorotation to simulate microgravity conditions.

2. To assess and compare the germination rates of maize seeds under clinorotation and normal gravity.

3. To analyze growth parameters, including shoot and root length, weight of the seeds after germination.

CHAPTER TWO

LITERATURE REVIEW

Clinorotation is a specialized technique used in space research to simulate microgravity conditions on Earth. It involves rotating biological samples continuously or semi-continuously around an axis perpendicular to the direction of gravity, resulting in a near-weightless environment for the samples. This technique allows researchers to study the effects of altered gravity on various biological processes, including plant growth, development, and cellular responses(Levine and Johnson, 1979).

The concept of clinorotation was first introduced in the 1960s as part of the broader efforts to understand the effects of microgravity experienced by astronauts during space missions. Researchers recognized the importance of studying the influence of gravity on living organisms to address the challenges posed by long-duration space travel and potential colonization of other celestial bodies. The utility of clinorotation in plant research became apparent as it provided an innovative tool to investigate plant responses to reduced gravity conditions. Traditional methods of simulating microgravity, such as parabolic flights and sounding rockets, had limitations in their duration and reproducibility. Clinorotation, on the other hand, offered a controlled and sustained microgravity simulation, making it suitable for conducting longer-term experiments(Paul and Ferl, 2019).

Several studies have utilized clinorotation to investigate plant growth and development under altered gravity conditions. For instance, researchers have examined seed germination, root growth, and shoot development in various plant species, including Arabidopsis, wheat, rice, and maize. By subjecting plants to clinorotation, scientists can gain insights into how altered gravity affects hormone regulation, gene expression, and other cellular processes(Sychev *et al.*, 2014).

2.1. GRAVITY

Gravity is a fundamental force that is expressed as the distance between two objects with respect to space. It pulls more strongly between objects with larger masses, It also weakens the farther apart objects are. Gravity is a ubiquitous force on earth, and every living organism is evolutionarily adapted and thrives in the presence of gravity. It is therefore not surprising that altered gravity environments such as hyper-gravity or microgravity have a significant effect on plants growth, development, and morphology (Malarvizhi *et al.,* 2021)

In our daily lives, we experience gravity as the force that keeps us on the surface of the Earth, and it is the reason why objects fall when dropped. The Earth's gravity is what gives us weight and allows us to walk on its surface. The Moon's gravity is what causes the tides on Earth, and the gravitational pull of the Sun is what keeps the planets in orbit around it. Gravity plays a crucial role in the formation and evolution of our universe. It is responsible for the formation of galaxies, stars, and planets. It also plays a role in the eventual fate of the universe, with gravity causing the eventual collapse of the universe into a black hole (Hawking, 1988).

2.2 MICROGRAVITY

Microgravity is the condition whereby people or objects appear to be weightless and experience no force of gravity. It is sometimes referred to as "zero gravity" But Micro means "very small", therefore this means that gravity is not totally absent but present in very small amount. The effects of microgravity can be observed when astronauts and objects are suspended in space. Microgravity can be experienced in other ways as well. For example, you can easily move heavy objects, astronauts can move devices weighing hundreds of pounds with their fingertips (NASA, 2017). Weightlessness, also known as microgravity, is the complete or near-complete absence of the sensation of weight. Weight is a measure of the force exerted on an object at rest in a strong gravitational field (such as on the earth's surface). The concept of microgravity was first explored during the early days of human spaceflight. The first human to experience microgravity was astronaut Yuri Gagarin, who orbited the Earth on April 12, 1961, since then microgravity has been a central theme of human space exploration and has provided researchers with a unique environment for studying the effects of gravity on various physical and biological processes.

With growing interest in space agriculture, scientists have been working for the last five decades to better understand the influence of microgravity on a variety of plants. As we explore deep into space, the logistics and economics of carrying packaged food to crew members become increasingly impractical. Besides, nutrients tend to deplete significantly in packaged food upon storage in space (Cooper *et al.*, 2017). Hence, plant biology experiments in space are necessary from the point of providing fresh food to the human crew and develop into sustainable bio-regenerative life support system (BLSS) for long-duration space exploration missions. For the last five decades, fascinating insights have been shed on plant biology through spaceflight studies aboard orbiting spacecraft, and through simulated microgravity platforms –

clinostat (2D and 3D), Rotating Wall Vessel (RWV) and Random Positioning Machine (RPM) on the ground.

2.3 LIVING SYSTEMS

Living systems are a fundamental aspect of the natural world, encompassing all living organisms and their interactions with each other and their environment. They are characterized by their ability to respond to changes in their surroundings, maintain homeostasis, and evolve over time. Living systems are studied in various fields such as biology, ecology, and systems science. One of the key properties of living systems is their ability to maintain homeostasis, which is the process of maintaining a stable internal environment. Homeostasis involves a range of mechanisms that help to regulate temperature, pH, and nutrient levels, among other things. For example, when the body temperature rises, humans and other animals will begin to sweat to cool themselves down. Plants also have mechanisms to regulate their internal environment, such as opening or closing their stomata to conserve water in response to changes in humidity (Miller and Spoolman, 2020; Tilman, 1997).

2.4. EFFECT OF MICROGRAVITY ON LIVING SYSTEMS

Microgravity, or the condition of almost zero-gravity, has profound effects on living systems, including humans, animals, plants, and microorganisms(Vandenbrink *et al.*, 2014). Previous studies have shown that changes in gravitational forces usually affect growth and development in plants as well as changes in the human body. Some of the notable effects of microgravity on living systems include;

1. Changes in bone density: In microgravity, there is no longer the force of gravity acting on the bones, leading to bone loss and decreased bone density. This can result in a higher risk of fractures and other bone-related problems (Miller and spoolman, 2020).

2. Muscle atrophy: Without the constant resistance provided by gravity, muscles in the body can weaken and atrophy. This can result in reduced strength and endurance (Massa *et al*., 2013)

3. Fluid shifts: In microgravity, fluids in the body tend to shift towards the upper body, causing fluid accumulation in the head and neck. This can lead to a puffy face, nasal congestion, and decreased visual acuity (LeBlanc *et al*., 2000; Massa *et al*., 2013)

4. Cardiovascular changes: The cardiovascular system can also be affected by microgravity, as blood no longer needs to be pumped against the force of gravity. This can result in a decrease in overall blood volume, changes in blood pressure, and altered heart function (Kiss *et al*., 2019; Hughson *et al*., 2016)

5. Immune system changes: The immune system can be affected by microgravity, leading to a decreased ability to fight infections and a higher risk of developing certain illnesses (Crucian *et al*., 2018)

6. Changes in growth and morphology: In the absence of gravity, plant roots no longer grow towards the ground, and shoot growth may also be affected. Plants grown in microgravity may have a different morphology compared to those grown on Earth. For example, they may appear more branched and bushy (Ferl and Paul, 2016; Kwon *et al*., 2019).

7. Changes in gene expression: Plants grown in microgravity may show altered gene expression patterns, which can affect their physiology and metabolism. For example, genes involved in stress responses and cell wall modification may be upregulated in microgravity (Correll *et al*., 2013; Hoson *et al*., 2014).

8. Changes in nutrient uptake: Plants grown in microgravity may have difficulty taking up nutrients, as they no longer experience the force of gravity which helps to pull water and nutrients towards their roots (Hoson *et al*., 2014).

9. Changes in response to light: In microgravity, the directional cues provided by gravity are absent, and this can affect how plants respond to light. For example, the leaves of some plants grown in microgravity may orient themselves perpendicular to the direction of light, rather than parallel as they would on Earth (Stutte *et al.*, 2006; Paul *et al*., 2012).

2.5 PLANTS

Plants are multicellular organisms that belong to the kingdom Plantae. They are characterized by their ability to use sunlight, carbon dioxide, and water to synthesize their own food through the process of photosynthesis. Plants consume CO2 which is a by-product of human metabolism, and then give off oxygen, which is needed for human survival. Photosynthesis is also very important, especially in driving the process for generating ATP, which humans require for metabolism (Papaseit *et a*l., 2000). Secondly, the primary producers in food chains are plants (Kering and Zheng, 2015), so much so that plants cannot be taken away from the human mechanism of survival. Plants can be found in a wide range of environments, from deserts and forests to oceans and wetlands. They are an important part of many ecosystems, providing food, shelter, and oxygen to other organisms, they come in a variety of sizes, shapes, and colors. Some are large trees that can live for hundreds of years, while others are small herbs that only live for a few months. They also have a wide range of adaptations that allow them to survive in different environments.

According to soga *et al.* (2002), humans who board any spacecraft will require metabolic energy for their bodies in addition to the energy required to power the spacecraft. Traveling through space could take years, and since humans are heterotrophs, resources such as oxygen for metabolic energy production would be needed in the form of food and in the process, these two forms of human sustenance-based space travel are mainly plant-based. Given the use of synthetic techniques, plants, for example, have long been regarded as the best oxygen generators, considering the use of synthetic methods (Stutte *et al*., 2002).

During space travel, one of the greatest effects on plants is the influence of gravity (Levine, 2010; Vandenbrink *et al*., 2014; Braun *et al.,* 2018; Kiss *et al*., 2019; Orukpe *et al*., 2021). This ubiquitous force usually influences plant development, productivity, and morphology at all levels, from the molecular level to the whole plant (Vandenbrink *et al*., 2014).

2.6 GROWTH RESPONSE OF PLANTS

The growth response of plants refers to the changes in the physical characteristics and developmental patterns of plants in response to various internal and external factors. These factors can be biotic (living organisms) or abiotic (non-living environmental factors). The growth response of plants is a dynamic process that allows them to adapt, survive, and thrive in their specific habitats. Some key factors influencing the growth response of plants include:

1. Light: Light is one of the most crucial factors affecting plant growth. Through the process of photosynthesis, plants convert light energy into chemical energy, enabling them to synthesize their own food. Different wavelengths of light can trigger specific growth responses, such as phototropism, which causes plant organs to grow towards or away from light sources.

2. Water: Adequate water availability is essential for plant growth. Water is required for various physiological processes, including nutrient uptake, cell expansion, and photosynthesis. Insufficient water can lead to wilting and stunted growth, while an excess of water can cause root rot and other problems.

3. Nutrients: Plants require a range of essential nutrients, such as nitrogen, phosphorus, potassium, and micronutrients, for healthy growth and development. Soil fertility directly impacts nutrient availability to plants, and deficiencies or imbalances can lead to various growth disorders.

4. Temperature: Temperature affects plant metabolism, enzyme activity, and other biochemical processes. Different plants have specific temperature ranges for optimal growth. Extreme temperatures, either hot or cold, can stress plants and hinder their growth.

5. Gravity: Gravitropism, as mentioned earlier, allows plants to respond to gravity by adjusting the growth of their roots and shoots. This response helps roots grow towards the soil for anchorage and nutrient uptake, while shoots grow upwards towards light.

1. Gravitropism (Geotropism):

Gravitropism also referred to as geotropism is the growth response of plants in relation to gravity. Plant roots show positive gravitropism, growing towards the gravitational pull (downwards), while shoots exhibit negative gravitropism, growing against gravity (upwards). This tropism is also regulated by auxin. In roots, auxin accumulates on the lower side when the plant is placed horizontally, promoting cell elongation on that side and causing the roots to grow downwards. In shoots, auxin accumulates on the upper side, inhibiting cell elongation and allowing the shoot to grow upwards (Sato *et al*., 2019)

Gravitropism is essential for plants to respond and adapt to their environment effectively. By growing roots deeper into the soil and shoots towards the light, plants can secure stable anchorage, access water and nutrients, and optimize photosynthesis for energy production (Geisler *et al*., 2014; Krieger *et al*., 2016).

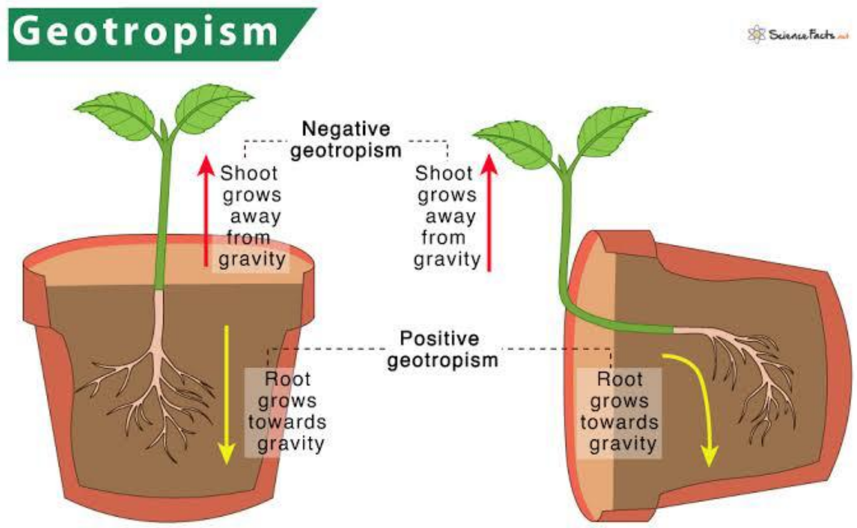


Figure 2.1: Geotropism (Geisler *et al.*, 2014)

2. Thigmotropism:

Thigmotropism is a specialized form of tropism exhibited by plants in response to mechanical stimuli or physical contact. Unlike phototropism or gravitropism, which involve responses to light and gravity, respectively, thigmotropism is a growth response to touch or mechanical pressure. This phenomenon allows plants to adapt and respond to their physical environment, helping them anchor themselves, climb, or react to external disturbances. Thigmotropic responses are commonly observed in climbing plants, vines, and tendrils. When a part of the plant, such as a tendril or stem, comes into contact with a solid support or another surface, it triggers a thigmotropic response. The plant responds by coiling, wrapping, or growing around the object it has touched. This behavior allows the plant to gain support and stabilize itself as it climbs upward, maximizing its exposure to light and optimizing photosynthesis (Telewski, 2006; Monshausen *et al.*, 2009).

The underlying mechanisms of thigmotropism are complex and involve both mechanical and biochemical processes. Thigmotropism is essential for the growth and survival of climbing plants and vines. By responding to physical contact, these plants can find suitable support structures for growth, avoid competing for space with other plants, and adapt to challenging environmental conditions. This growth response enables them to take advantage of the vertical space, access better light conditions, and improve their reproductive success (Gagliano *et al.*, 2014).

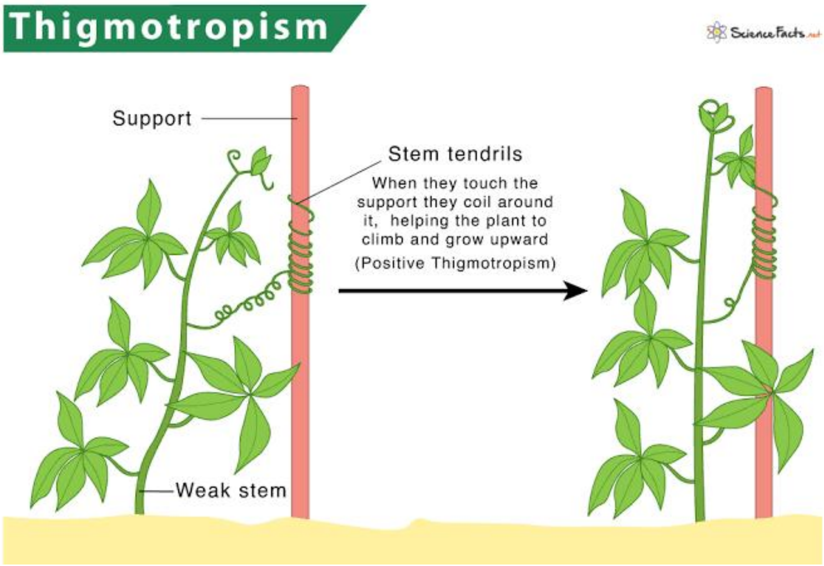


Figure 2.2: Thigmotropism (Telewski, 2006)

3. Hydrotropism

Hydrotropism is a plant growth response that involves the directional movement of plant roots towards or away from water gradients in the soil. This phenomenon allows plants to efficiently navigate their root systems towards regions with higher water availability, ensuring their survival and optimal water uptake. Hydrotropism complements other tropic responses, such as gravitropism and phototropism, by enabling plants to respond specifically to water gradients in their environment (Toyota and Gilroy, 2013).

Hydrotropism is of great significance to plants, especially in arid or unevenly watered environments. By actively growing their roots towards water-rich regions, plants can enhance their water uptake efficiency and avoid areas of water stress. Additionally, hydrotropism contributes to root exploration and spatial distribution in the soil, enabling plants to occupy a diverse range of ecological niches(Takahashi *et al.*, 2003).

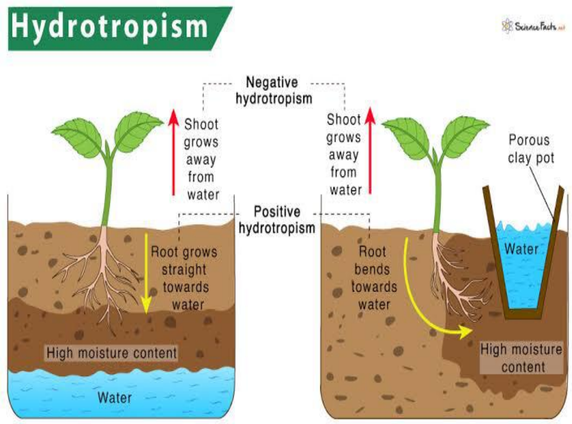


Figure 2.3: Hydrotropism (Toyota and Gilroy, 2013).

4. Chemotropism

Chemotropism is a type of tropism exhibited by some plants and fungi in response to chemical stimuli. Unlike phototropism and gravitropism, which respond to light and gravity, respectively, chemotropism involves directional growth responses to specific chemical cues in the environment. This phenomenon allows organisms to move towards or away from particular chemical substances, depending on their biological needs (Tsung-Hsun Tsao, 1949) Chemotropism can be observed in various contexts:

1. Pollen Tube Growth: In flowering plants, the process of fertilization involves the growth of a pollen tube down the style towards the ovary. This growth is guided by chemotropism, as the tube is attracted towards chemicals secreted by the ovule, ensuring successful fertilization.

2. Root Growth and Nutrient Acquisition: Plant roots can exhibit chemotropism to navigate towards sources of nutrients or ions in the soil. For example, the roots of plants can sense and grow towards higher concentrations of essential minerals like nitrate, phosphate, or iron.

3. Fungal Hyphae Growth: Fungi, especially those that form mycorrhizal associations with plant roots, use chemotropism to extend their hyphae towards root exudates, where they can form symbiotic relationships and exchange nutrients with the host plant.

Chemotropism plays a vital role in the survival, growth, and reproduction of plants and fungi by enabling them to locate essential resources or interact with beneficial partners in their environment. This complex response to chemical cues demonstrates the sophisticated ways in which organisms have evolved to interact and adapt to their surroundings.

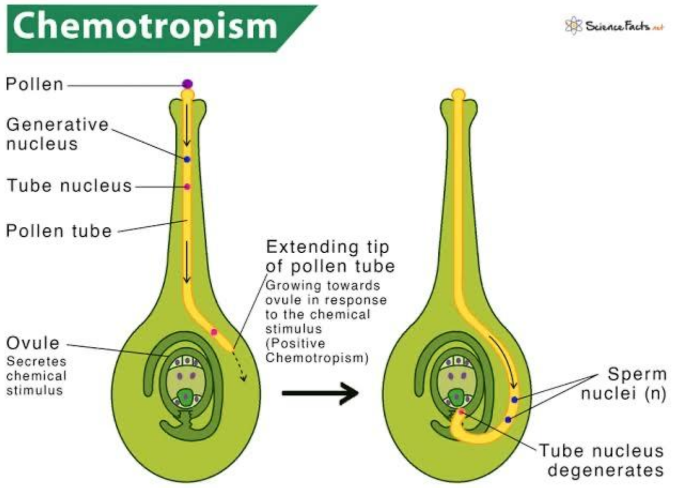


Figure 2.4: Chemotropism (Tsung-Hsun Tsao, 1949)

Tropisms are vital for plants to adapt to their surroundings and optimize their growth in response to changing environmental conditions. These growth responses are often coordinated by plant hormones, particularly auxins, which play a crucial role in regulating cell elongation and growth direction. Tropisms are essential for plants to find light for photosynthesis, anchor themselves in the soil, and navigate through their environment to survive and reproduce successfully.

2.7. PLANTS RESPONSE TO MICROGRAVITY

The plant develops under the influence of a single factor, earth's gravity, 1g. Any change in plant growth, development, and morphology can make them highly inflammatory, resulting in dramatic changes in cell proliferation, development, and morphology at all levels, from cellular, molecular to tissue, and the whole plant level (vandenbrink *et al.*, 2014). Plants exposed to altered gravity (microgravity or hyper gravity) respond in three distinct ways. One, cells' perception of gravity, two, the transformation of that viewed altered gravity into action, and finally, cells' reaction to change in gravity. On many occasions, plants of different species have been successfully grown in space, under microgravity conditions, and at different developmental stages, including the completion of the full seed-to-seed life cycle (Merkys *et al.*, 1984; Musgrave *et al.*, 2000; Karahara *et al.*, 2020). In parallel, similar plant growth experiments on Earth, using ground-based facilities for microgravity simulation, such as clinostats and random positioning machines have been successfully run (Kiss *et al.*, 2019). They have provided a reliable replica of the biological results obtained in space experiments under real microgravity, even though the gravity vector is not possible to be avoided or removed on the Earth surface (Herranz *et al.*, 2013; Medina *et al.*, 2015; Van Loon, 2016).

2.8. SELECTED CROPS FOR MICROGRAVITY SIMULATION

Selecting crops for microgravity simulations is an important consideration for future space missions or long-duration stays on other celestial bodies. The choice of crops must take into account factors like nutritional value, growth cycle, resource requirements, and ease of cultivation in a microgravity environment, these crops are selected because of their nutritional and economical values. The selection of crops for microgravity simulations or space farming experiments may vary depending on the research being conducted and the specific goals of the mission. However, some of the crops that have been studied for space agriculture includes:

1. Lettuce (*Lactuca sativa*): Lettuce is a popular candidate for space farming due to its short growth cycle, small space requirements, and high nutritional value. It is a leafy green vegetable rich in essential vitamins and minerals, including vitamin C, vitamin K, and folate. NASA and other space agencies have conducted multiple experiments on the International Space Station (ISS) to grow lettuce successfully in microgravity conditions (Massa and Wheeler, 2017).
2. *Arabidopsis thaliana* (Mouse-ear cress): *Arabidopsis thaliana* is a small flowering plant commonly used as a model organism in plant biology research. Its small size and rapid growth cycle (around 6 weeks from seed to seed) make it suitable for microgravity experiments. *Arabidopsis* has been extensively studied on the ISS and other space missions to understand the effects of microgravity on plant growth, development, and gene expression. Insights gained from studying Arabidopsis can also be applied to other crops (Kwon and Sparks, 2020).
3. Soybean (*Glycine max*): Soybean is a valuable crop due to its high protein content and versatility in various food products. It has been studied in space environments to evaluate its adaptability to microgravity and its potential as a protein-rich food source for astronauts during long-duration missions. NASA has conducted experiments with soybeans on the ISS as part of their efforts to develop sustainable space farming systems (Paul and Ferl, 2020).
4. Peanut (*Arachis hypogaea*): Peanuts have been considered for space farming due to their

nutritional value, especially as a source of protein and healthy fats. They are also relatively easy to grow and can fix nitrogen in the soil, which is beneficial for space farming systems (Miyazawa *et al.,* 2022)

5. Watermelon (*Citrullus lanatus*): Watermelon is a good candidate for space missions because of its high-water content, which can contribute to the hydration and sustenance of astronauts. Additionally, it contains essential vitamins and minerals (Correll *et al*., 2014)
6. Maize (*Zea mays*): Corn has been studied for its adaptability to space environments and its potential as a staple crop. It is a high-yielding plant and can be used for food, animal feed, and industrial purposes (Wheeler *et al*., 2017)
7. Cucumber (*Cucumis sativus*): Cucumbers are relatively fast-growing and space-efficient plants, making them suitable for space farming. They are a good source of hydration and certain nutrients (Paul *et al*., 2020)
8. Carrot (*Daucus carota*): Carrots are rich in vitamins and antioxidants, making them a valuable addition to the diet of astronauts on long-duration missions (Yamashita *et al*., 2016)
9. Tomato (*Solanum lycopersicum*): Tomatoes are rich in vitamins and can be used in various dishes, adding variety to astronauts' diets. They have been studied for space farming due to their potential for cultivation in confined spaces (Johnson *et al*., 2018)

These crops have been selected for their potential to provide essential nutrients, their ability to adapt to microgravity conditions, and their suitability for space farming systems. By studying and successfully growing these crops in simulated microgravity conditions, we can better understand the challenges and opportunities of agriculture in space (Vandenbrink *et al*., 2016)

2.9. MAIZE (*Zea Mays*) as a crop for microgravity simulation

Maize, scientifically known as *Zea mays*, is one of the most important cereal crops in the world. It is a member of the grass family Poaceae and is native to the Americas, where it has been cultivated for thousands of years. Maize is widely grown for its edible seeds, which are commonly known as corn, and it serves as a staple food for millions of people worldwide. Apart from its significance as a food crop, maize also finds diverse applications in industries, animal feed, and biofuel production (Paul *et al.*, 2012). Maize (*Zea mays*) is a valuable crop for studying plant growth and development in microgravity simulations. It has been used in various spaceflight experiments and ground-based studies to understand how plants respond to altered gravitational conditions.

Some reasons why maize is a suitable crop for microgravity simulations include:

1. Robust Growth: Maize is a fast-growing and hardy plant, making it suitable for conducting experiments with short growth cycles. This allows researchers to observe multiple generations of maize plants in a relatively short period, facilitating the study of heritable traits and responses.
2. Easy to Manipulate: Maize is easy to grow and manipulate in laboratory settings. Researchers can control various environmental factors, such as light, temperature, and nutrient availability, to study specific aspects of plant growth and development in microgravity.
3. Economical and Nutritious: As a major staple crop worldwide, maize is an economically important plant. It also provides a nutrient-rich food source, making it relevant for space agriculture and potential food production during long-duration space missions.

4. Demonstrated Adaptability: Previous spaceflight experiments with maize have shown its ability to adapt to microgravity conditions. These studies have provided insights into the changes in gene expression, hormone levels, and growth patterns in maize plants exposed to space environments.

5. Practical Applications: Understanding how maize responds to microgravity has practical applications for space agriculture and the potential future colonization of other planets. Maize can be an essential component of closed-loop life support systems, contributing to oxygen generation, carbon dioxide removal, and food production.

By using maize as a crop for microgravity simulations, researchers can gain valuable insights into the fundamental mechanisms of plant growth and adaptation to altered gravitational conditions. The knowledge gained from studying maize in microgravity can also contribute to advancements in agricultural practices on Earth, especially in challenging environments or areas with limited resources. Overall, maize serves as an excellent model organism to study plant responses to microgravity, and its versatility and importance as a crop make it a valuable asset for space research and agriculture.

Effects of Clinorotation on Maize

Clinorotation induces changes in various aspects of maize plant growth and development:

a. Root Growth: Under clinorotation, the orientation of the gravity vector is disrupted, leading to changes in root growth direction and behavior. Studies have shown altered root growth patterns in response to simulated microgravity (Soga *et al.*, 2016)

b. Shoot Morphology: The shoot system of maize also experiences modifications under clinorotation, with differences observed in stem elongation, leaf development, and branching (Soga *et al*., 2016)

c. Hormonal Changes: Gravity plays a role in the distribution of plant hormones. Clinorotation studies have revealed changes in hormonal levels, affecting physiological processes such as cell division and differentiation (Soga *et al*., 2016)

d. Gene Expression: Altered gravity conditions influence gene expression patterns in maize, leading to changes in the regulation of various genes involved in growth and stress responses (Soga *et al*., 2016)

Benefits to Health

Research on maize using clinorotation has broader implications for human health:

a. Space Agriculture: Understanding how maize responds to microgravity conditions can aid in developing sustainable space agriculture, ensuring a reliable food source for future astronauts during extended space missions (Paul *et al*., 2012)

b. Biomedical Research: The insights gained from studying maize's response to clinorotation can provide valuable information about how altered gravity affects living organisms, including humans. This knowledge can be relevant for mitigating health issues associated with extended space travel or bed rest (Wolverton *et al*., 2014)

CHAPTER THREE

MATERIALS AND METHODS

3.1 STUDY SITE

The experiment was conducted at the Space-Earth Environment Research Laboratory, University of Benin, under the Centre for Atmospheric Research of the National Space Research and Development Agency (NASRDA).

3.2. SELECTED CROP COLLECTION

The maize seeds were obtained from the seed bank of Space-Earth environment research laboratory, Benin City, Edo State, Nigeria.

Plate 1: Maize seeds located at the seed bank of Space-Earth environment research laboratory

photo credit: (Alex Orukpe)

3.3. APPARATUS

The following apparatus were used during the course of the experiment and they include:

Petri dish, Beakers, measuring cylinder (100ml and 1000ml), spatula and forceps.

3.4. EQUIPMENT

The following equipment were used during the experiment and they include:

Microscope, 2D clinostat, and digital weighing balance.

Figure 3.1: 2D clinostat (Orukpe *et al*., 2021)

3.5. MATERIALS AND REAGENTS

The following materials were used in the course of the experiment been carried out. They include; Maize seeds, distilled water, Agar Agar, and growth stimulators; 100ppm and 500ppm of Indole acetic acid (IAA), 100ppm and 500ppm Sodium nitroprusside (SNP), tape, cotton wool, crystal violet.

3.6. EXPOSURE OF SEEDS TO WATER

In a beaker containing 20mls of distilled water, 40 maize seeds were soaked for a period of one hour. After the desired time was attained, decanted from the solution and placed on a clean filter paper to air dry, according to a relevant method.

3.7. EXPOSURE OF SEEDS TO GROWTH STIMULATORS

3.7.1. Preparation of 100ppm of Indole acetic acid (IAA)

In the preparation of 100ppm of IAA, 0.025g of the IAA is weighed and dissolved in 250ml of distilled water, shake properly for complete dissolution.

3.7.2. Preparation of 500ppm of Indole acetic acid (IAA)

In the preparation of 500ppm of IAA, 0.125g og the IAA is weighed and dissolved in 250ml of distilled water and Shaked properly for complete dissolution.

3.7.3. Preparation of 100ppm of Sodium nitroprusside (SNP)

In the preparation of 100ppm of SNP, 0.025g of the SNP is weighed and dissolved in 250ml of distilled water and Shaked properly for complete dissolution.

3.7.4. Preparation of 500ppm of Sodium nitroprusside (SNP)

In the preparation of 500ppm of SNP, 0.125g of the SNP is weighed and dissolved in 250ml of distilled water and Shaked properly for complete dissolution.

After the preparation of the growth stimulator the maize seeds is then subjected to the growth stimulators for a period of 1hour before decanting and placing on a clean filter paper and allowed to air dry.

3.8. AGAR PREPARATION

Agar was prepared, following standard methods, according to Orukpe *et al.* (2021) 100 mL of 1-1.5% Duchefa Biochemie Plant Agar-Agar in tap water (1.5 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. Two petri dishes were filled with 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 a bit.

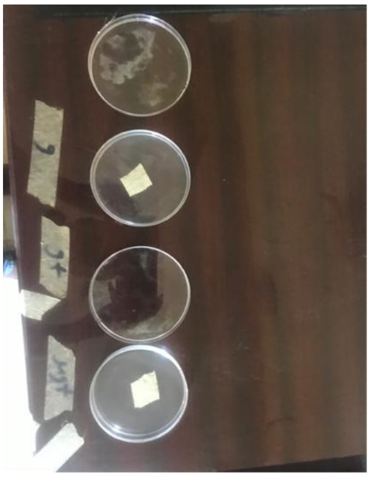


Plate 2: The prepared agar

photo credit: (Alex Orukpe)

3.9. SOWING THE MAIZE SEEDS

The maize seeds were weighed individually using a digital weighing balance, Model No. NBT-A200, with an average weight of each seed to be 0.29g. In each petri dish, twenty seeds of each accession were planted on the agar-agar by using the tweezers in the same direction in order to identify the micropyle.

Plate 3: Maize seeds sown into the cooled agar

photo credit: (Alex Orukpe)

3.10. SUBJECTING TO CLINOROTATION

After seeding the seeds on the agar-agar surface, one of the petri dishes were placed vertically using a petri dish holder as control and the second petri dish was mounted on the clinostat. The clinostat was rotated at a speed of 0.5rpm and 2.5rpm for 120 hours (5 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 experiment was prepared in several batches with the different growth stimulators and subjected to the clinostat at different rotation of 0.5rpm and 2.5rpm and was studied for a period of 120 hours, while the control seeds were placed on the laboratory benches for the same period of time and observations were taken every 24 hours, to observe for germination.

Plate 4: maize seeds subjected to clinorotation in a simulated microgravity environment using a clinostat and maize seeds subjected to normal gravity

photo credit: (Alex Orukpe)

3.11. GERMINATION PROPERTIES

Several plant germination parameters were measured, including the time for the first germination, time for the last germination, germination percent at 72hrs, root length, shoot length, the number of prominent roots and the number of branches. All seed germination parameters were calculated according to a relevant protocol (AOSA, 1983).

3.11.1. TIME FOR THE FIRST GERMINATION

The time for the first germination refers to the moment when the very first seed in a batch begins to sprout. This is an important event because it marks the initiation of the germination process (Musgrave *et al*., 2000).

3.11.2. TIME FOR THE LAST GERMINATION

The time for the last germination refers to the point in time when the final seed in a batch successfully germinates. This indicates the completion of the germination process for that specific batch of seeds (Musgrave *et al*., 2000).

3.11.3. FIRST GERMINATION PERCENT

The "first germination percentage" typically refers to the percentage of seeds from a batch that successfully germinate for the first time (Musgrave *et al*., 2000).

3.11.4. GERMINATION PERCENT AT 72HOURS

Germination percentage refers to the proportion of seeds from a given batch that successfully sprout and begin to grow. It's a measure of the viability of the seeds and is often used by gardeners, farmers, and researchers to assess seed quality. Higher germination percentages generally indicate healthier and more viable seeds, which is important for successful plant propagation (Musgrave *et al*., 2000).

3.11.5. TIME SPREAD OF GERMINATION

The time spread of germination refers to the range of time it takes for seeds in a batch to begin germinating. In other words, it's the difference between the earliest and latest germination times among the seeds (Musgrave *et al.*, 2000).

3.11.6. MEDIAN GERMINATION TIME

The median germination time is the point in time by which half of the germinated seeds have sprouted. In other words, it's the middle value in the sequence of germination times. It gives you an idea of when most of the seeds in a batch are likely to have started sprouting (Musgrave *et al.*, 2000).

3.11.7. FINAL GERMINATION PERCENT

The "final germination percentage" refers to the percentage of seeds in a batch that eventually germinate after a certain period of time, often until no more seeds are likely to sprout. This measurement takes into account all the seeds that have germinated over the entire germination period (Musgrave *et al.*, 2000).

3.11.8. MEAN DAILY GERMINATION

The "mean daily germination" refers to the average number of seeds that germinate each day over a certain period of time. To calculate the mean daily germination, you would divide the total number of germinated seeds by the number of days it took for them to germinate (Musgrave *et al.*, 2000).

3.11.9. GERMINATION INDEX

The "germination index" is a numerical value that indicates the relative vigor or quality of seeds based on their germination performance. It's often calculated using the formula:

$$\text{Germination Index} = (\text{Number of Normal Seedlings} / \text{Total Number of Seeds}) \times 100$$

In this formula, "Number of Normal Seedlings" refers to the seeds that have successfully germinated and developed into healthy, viable seedlings(Musgrave *et al*., 2000).

3.11.10. PEAK PERIOD OF GERMINATION

The "peak period of germination" refers to the specific time frame during which the highest number of seeds in a batch begin to germinate. This period often signifies optimal conditions for germination, such as ideal temperature, moisture, and light(Musgrave *et al*., 2000).

CHAPTER FOUR

4.1 RESULTS

Figure 4.1: Germination of maize seeds after 5 days

photo credit: (Alex Orukpe)

Figure 4.2: maize under microgravity

photo credit: (Alex Orukpe)

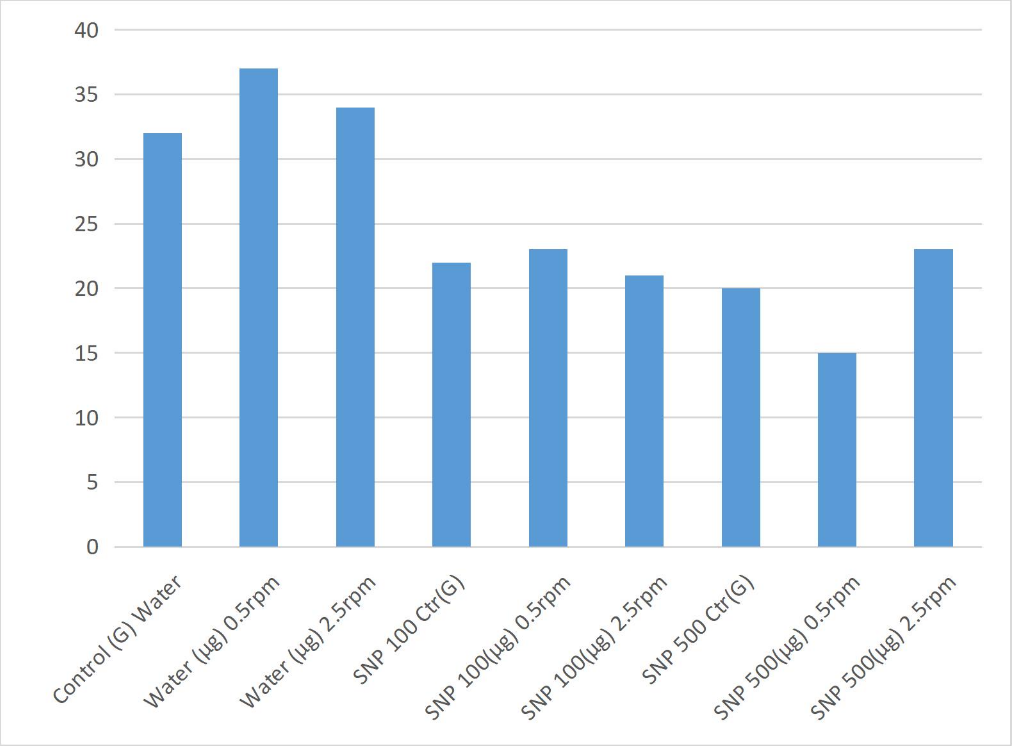
Figure 4.3: maize under gravity

photo credit: (Alex Orukpe)

Table 4.1: Germination indices of the maize seeds subjected to clinorotation

Use the GPs for graphs	Time for the first germination (h)(T0)	Time for the last germination (h) (Tg)	First Germination percent	Germination percent (%) at 72 hrs	Time spread of germination or Germination distribution (Tg-To) (h)	Median germination time (T50) (hrs)	Final Germination Percent, FGP	Mean Daily germination (%/h)	Germination index	Peak period of germination or Modal time of germination
Control (G) Water	32	81	12.8	75	49	55	100	1.23	2.51	45.6
Water (µg) 0.5rpm	37	65	21.3	100	28	63	100	1.54	2.60	44.2
Water (µg) 2.5rpm	34	84	17.0	65	50	62	100	1.19	2.51	44.2
SNP 100 Ctr(G)	22	100	4.3	80	78	39	100	1.00	2.78	22.1
SNP 100(µg) 0.5rpm	23	51	12.8	100	28	38	100	1.96	2.87	22.8
SNP 100(µg) 2.5rpm	21	50	17.0	100	29	29	100	2.00	2.93	22.8
SNP 500 Ctr(G)	20	98	25.5	75	78	46	100	1.02	2.85	45.6
SNP 500(µg) 0.5rpm	15	75	21.3	90	60	35	100	1.33	2.85	21.8
SNP 500(µg) 2.5rpm	23	51	34.0	100	28	27	100	1.96	3.03	22.8
IAA 100 Ctr(G)	16	74	51.0	95	58	22	100	1.35	3.14	22.8
IAA 100(µg) 0.5rpm	15	50	55.3	100	35	21	100	2.00	3.14	21.6
IAA 100(µg) 2.5rpm	23	50	8.5	100	27	36	100	2.00	2.86	21.6
IAA 500 Ctr(G)	22	74	12.8	90	52	34	100	1.35	2.86	22.6
IAA 500(µg) 0.5rpm	16	49	17.0	100	33	32	100	2.04	2.93	23.3
IAA 500(µg) 2.5rpm	23	76	8.5	85	53	28	100	1.32	2.85	21.8

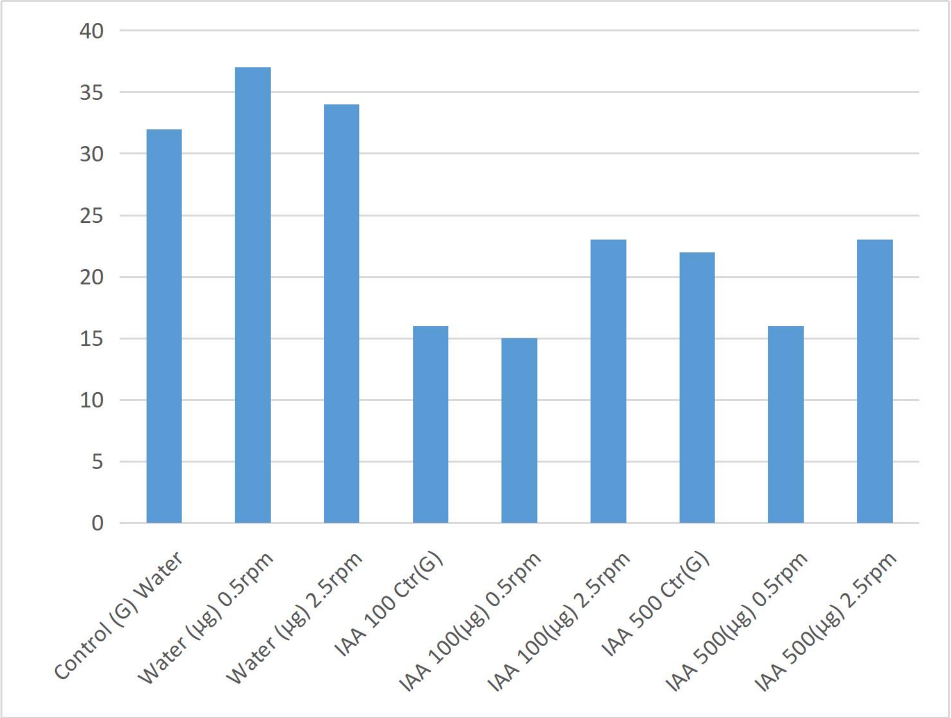
KEYS: SNP- Sodium Nitroprusside; IAA- Indole-3-Acetic Acid; G- Gravity; µg- Microgravity; rpm- Revolution per minute



Time of first germination (hr)

Figure 4.4: The amount of time for maize seeds to show the first instance of germination, following exposure to gravity and micro-gravitational forces

KEYS: SNP- Sodium Nitroprusside; G- Gravity; µg- Microgravity; rpm- Revolution per minute



Time of first germination (hr)

Figure 4.5: The amount of time for seeds to show the first instance of germination, following exposure to gravity and micro-gravitational forces

KEYS: IAA- Indole-3-Acetic Acid; G- Gravity; µg- Microgravity; rpm- revolution per minute

Table 4.2: Growth parameters of the maize seeds at 5 days after sowing

Use the GPs for graphs	No. of Root	No. of Branch	Root length (cm)	Shoot length (cm)	Fresh wt. (g)	Dry Wt. (g)
Control (G) Water	7	8	3.70	5.53	0.92	0.32
Water (µg) 0.5rpm	11*	25*	3.76	5.10	1.92*	0.59*
Water (µg) 2.5rpm	13*	9	5.00	5.53	0.74	0.23
SNP 100 Ctr(G)	4	9	2.10	2.20*	0.52*	0.16
SNP 100(µg) 0.5rpm	5	8	3.00	5.20	0.93	0.29
SNP 100(µg) 2.5rpm	7	25*	6.00*	8.10*	0.79	0.25
SNP 500 Ctr(G)	3*	8	3.00	3.50	0.55*	0.17
SNP 500(µg) 0.5rpm	6	9	3.10	5.50	0.74	0.23
SNP 500(µg) 2.5rpm	4	9	4.60	5.10	0.63	0.24
IAA 100 Ctr(G)	4	10	1.80	2.40*	1.09	0.20
IAA 100(µg) 0.5rpm	13*	6	2.50	5.00	1.30*	0.54
IAA 100(µg) 2.5rpm	20*	13*	13.93*	5.87	2.04	0.70*
IAA 500 Ctr(G)	5	9	2.17	4.00	0.64	0.26
IAA 500(µg) 0.5rpm	11*	13*	2.40	6.27	0.70	0.25
IAA 500(µg) 2.5rpm	14*	8	2.50	4.63	0.66	0.45
p-value	0.019	0.025	0.043	0.221	0.069	0.106
LSD (0.05)	4	5	2.06	2.13	0.35	0.18

KEY: CTRL- control; IAA- Indole-3-Acetic acid; SNP- Sodium Nitroprusside; RPM- revolution per minute; PPM- part per minute; µg- microgravity; G- gravity

Table 4.3: Catalase value in shoot, root and seeds of maize subjected to clinorotation

	Shoot		Root		Seed		p-value
	CAT(Mg)	CAT (g)	CAT(Mg)	CAT (g)	CAT(Mg)	CAT(g)	
CTRL 0.5RPM(Water)	13.86	13.49	13.48	13.63	13.99	13.43	0.632
CTRL 2.5RPM(Water)	13.62	13.49	13.73	13.63	13.91	13.43	0.424
(IAA100ppm) 0.5RPM	40.33	41.8	41.36	41.25	42.43	42.05	0.137
(IAA 100PPM) 2.5RPM	42.89	41.8	42.43	41.25	42.92	42.05	0.662
(IAA 500PPM) 0.5RPM	40.33	40.49	39.57	39.74	41.2	40.55	0.742
(IAA 500PPM) 2.5RPM	39.43	40.49	38.86	39.74	39.9	40.55	0.114
(SNP100PPM) 0.5RPM	16.01	17.31	16.25	16.22	16.49	16.91	0.124
(SNP100PPM) 2.5RPM	21.84	15.24	21.64	16.22	21.98	16.91	0.035
(SNP500PPM) 0.5RPM	21.96	22.46	22.09	21.99	20.13	23.46	0.253
(SNP500PPM) 2.5RPM	22.03	22.46	22.29	21.99	23.4	23.46	0.134

KEY:CAT- catalase; CTRL- control; IAA- Indole-3-Acetic acid; SNP- Sodium Nitroprusside; RPM- revolution per minute; PPM- part per minute; Mg- microgravity; g- gravity

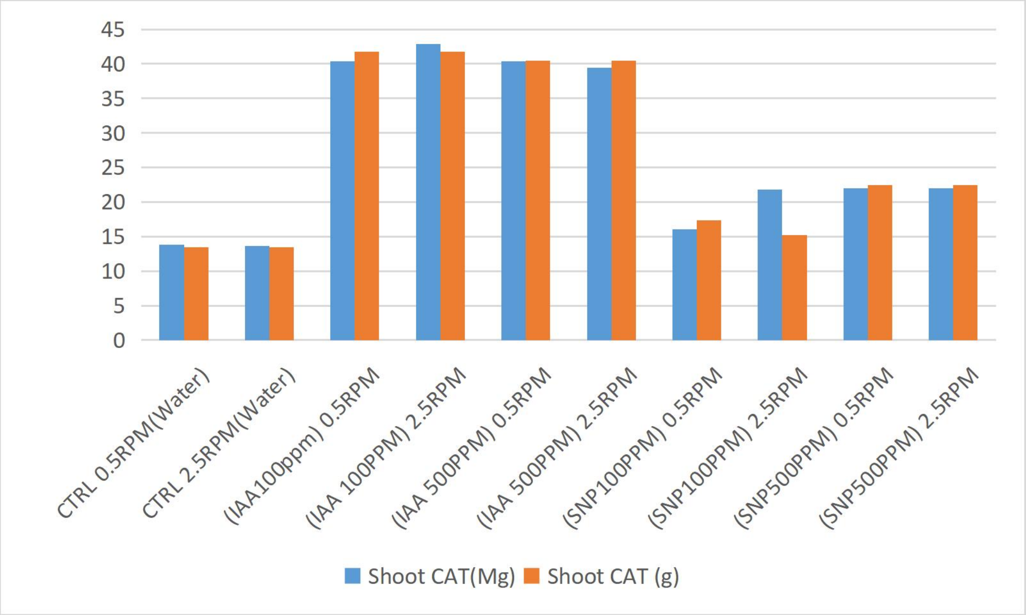


Figure 4.6: catalase value for the shoot

KEY: CAT- catalase; CTRL- control; IAA- Indole-3-Acetic acid; SNP- Sodium Nitroprusside; RPM- revolution per minute; PPM- part per minute; Mg- microgravity; g- gravity

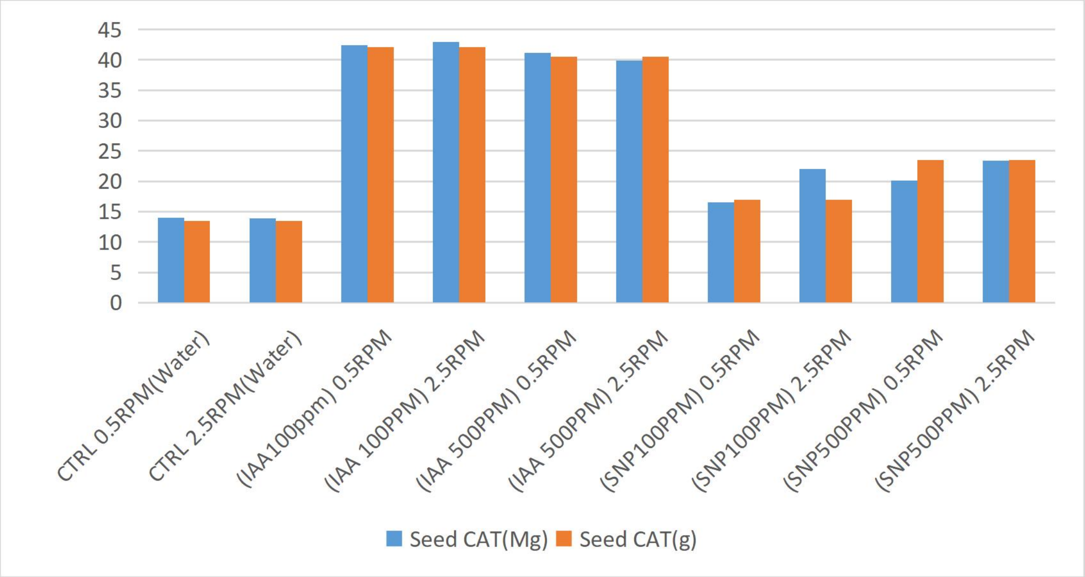


Figure 4.7: Catalase value for the seeds

KEY: CAT- catalase; CTRL- control; IAA- Indole-3-Acetic acid; SNP- Sodium Nitroprusside; RPM-revolution per minute; PPM- part per minute; Mg- microgravity; g- gravity

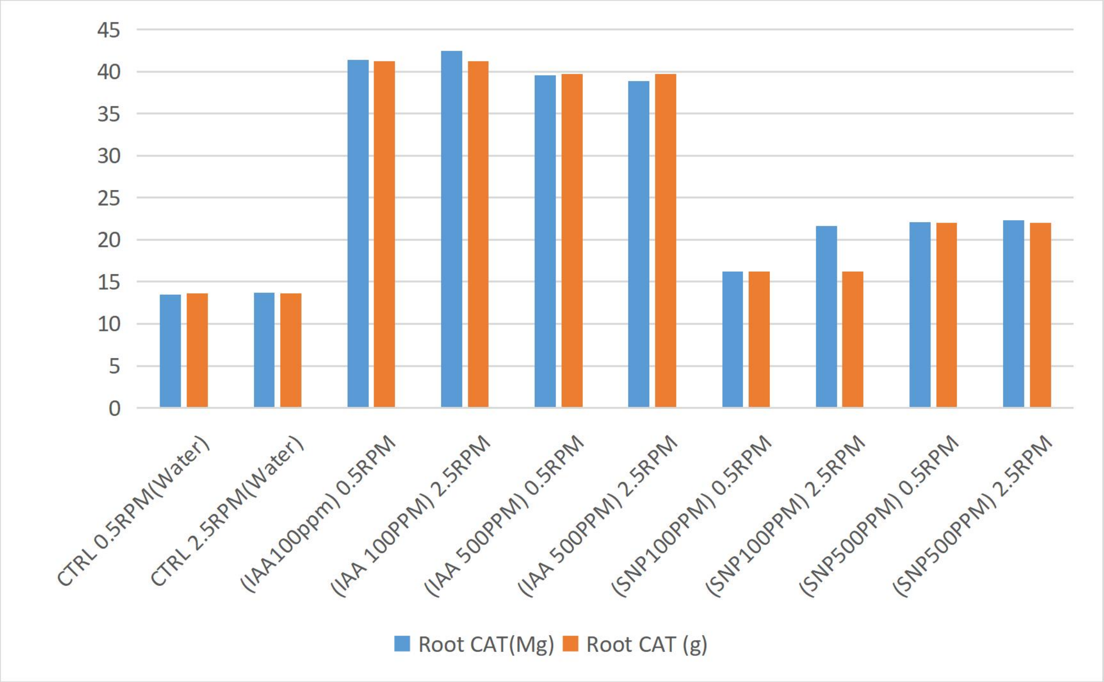


Figure 4.8: Catalase value for the root

KEY: CAT- catalase; CTRL- control; IAA- Indole-3-Acetic acid; SNP- Sodium Nitroprusside; RPM- revolution per minute; PPM- part per minute; Mg- microgravity; g- gravity

CHAPTER FIVE

DISCUSSION

Plate 1, 2 and 3 shows the germination of the maize after 5 days, plate 2 shows the germination of maize seeds under clinorotation and plate 3 shows the germination of maize seeds on earth gravity. Base on physical observations it was observed that the maize seeds under clinorotation germinated first, was more vegetative and also had more growth compared to the one on earth gravity. In the absence of gravity, the plant roots no longer grew towards the ground, and shoot growth were also affected(Monje *et al.,* 2003).

Table 1 presents germination indices for different treatments. The treatments include Control (G) with water, Water (µg) at different rotations per minute (rpm), SNP (Sodium Nitroprusside) at different concentrations and rotations per minute, and IAA (Indole-3-Acetic Acid) at different concentrations and rotations per minute. It also provided various germination indices such as the time for the first germination (T0), time for the last germination (Tg), first germination percent, germination percent at 72 hours, final germination percent (FGP)(Ikhajiagbe *et al*., 2023).

According to the table it was observed that the control plates without treatment (control with water) took longer time to germinate for both the plate under microgravity and gravity while the plates treated with growth stimulators germinated first, With this it shows that the growth stimulators increased the growth rate of the maize thereby making the maize seeds germinate faster.

For the different concentration of the growth stimulators (both Sodium Nitroprusside and

Indole-3-Acetic Acid) at the different rotation per min (rpm) it was observed that the time of first germination and time for last germination are different, with the plate treated with 500ppm of SNP under microgravity at a rotation level of 0.5rpm and the plate treated with 100ppm of IAA at 0.5rpm germinating first at 15hours after clinorotation started, therefore this would start photosynthesizing before others, While the control plate with water at a rotation level of 0.5rpm was observed to have germinated last.

Table 2 shows the germination parameters of the maize seeds after germination, this parameters include the number of roots, number of shoots, root lengths, shoot lengths and others. According to the table, it shows that all plates subjected to clinorotation at the different rotation level have the highest number of root, shoots, the lengths of the roots and shoots. This means that the microgravity environment increased the rate of crop development(Paul *et al.*, 2012)

Plants are susceptible to stress, plants grown in microgravity environments, may experience stress due to changes in gravity, light, and other environmental factors. Some of the main stressors that plants may encounter in microgravity include changes in the direction and intensity of light, altered gravity-sensing and growth, These stressors can affect the growth, development, and overall health of the plant. These plants then produces a stress hormone which allows them to adapt to the changing environmental conditions one of which is catalase(CAT). Catalase is an enzyme that is commonly found in most living organisms and is responsible for catalyzing the breakdown of hydrogen peroxide a toxic by-product of cellular metabolism into water and oxygen. It plays an important role in protecting cells from oxidative stress, as it functions as a stress hormone in plants, it is known to be rapidly induced by various stressors, including microgravity, heat, drought, salt, and heavy metal stress. when a plant is been grown under microgravity environment(clinostat) the plants experiences stress due to microgravity, catalase(CAT) acting as a stress hormone in plants helps the plant to overcome these stress thereby increasing it's growth rate and productivity. Table 3 shows how catalase overcame the stress posed by microgravity environment with an increase in the length of the shoot, root and seed.

CONCLUSION

This research conducted on the determination of germination parameters of maize subjected to clinorotation indicates that clinorotation, a technique simulating microgravity conditions, has a significant impact on the germination process of maize seeds. The study showed that clinorotation affected various germination parameters such as germination rate, percentage of germination, root and shoot growth, and overall seedling development. These findings provided valuable insights into the potential effects of microgravity on plant growth and could have implications for future space agriculture or controlled environment agriculture systems.

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