

Minireview Article

EXPLORING MICROGRAVITY'S EFFECT ON SEED GERMINATION AND GROWTH OF PLANTS

ABSTRACT

The study of the effects of microgravity on plant cells has gained significant attention, especially in the context of space experiments, which necessitates a comprehensive understanding of plant growth and development in altered gravitational conditions. There is a historical evolution of microgravity research on plant growth, tracing its origins from early experiments on V-2 rockets to the advanced facilities on the International Space Station. Understanding the impact of microgravity on seed germination, seedling establishment, and overall plant development is essential for future space agriculture and the development of sustainable food production systems. In microgravity, plants encounter unique physical and physiological challenges, making it essential to explore the physiological and biochemical responses of plant cells to this environment. Various factors contribute to the alterations observed in plant behavior under microgravity, including changes in water and nutrient distribution, disruptions in hormone signaling, and modifications in the expression of secondary metabolites. In particular, microgravity resulted in delayed germination, reduced water uptake, and altered growth orientations in the absence of gravity. Physiological and biochemical in plants due to microgravity caused by the influence on the phytochrome system, gas exchange photosynthesis, and the production of secondary metabolites. Microgravity affects mechanocellular responses of plants which elucidates how the absence of gravity can disrupt cytoplasmic streaming, impact cell wall rigidity, and cause damage to cell membranes. These alterations in plant cell behavior under microgravity conditions can have profound implications for plant growth and development. Space farming faces challenges and constraints of cultivating plants in space, such as managing water distribution, temperature fluctuations, and radiation exposure, are addressed. Innovative methods for planting crops in space, including plant pillows, greenhouse cultivation, and soil-less systems, are discussed.

In the future, research efforts should focus on selecting or genetically modifying plant varieties with enhanced tolerance to the diverse stress encountered in space environments. Thus, it is concluded that space farming underscores the importance of comprehensive studies on plant responses to microgravity, offering valuable insights for both space exploration and terrestrial agriculture.

1. INTRODUCTION

The physiological and biochemical responses in plant cells under varying gravitational conditions have recently gained increased attention, particularly in ground-

based investigations that have paved the way for space experiments. Therefore, it is imperative to acquire a comprehensive comprehension of the processes involved in plant growth and development in microgravity environments (Hassanpour and Abdel Latef, 2023).

Gravity, known as the force that draws two objects together, is accountable for the weight of an object on a planetary surface. On Earth, objects experience an acceleration of approximately 9.81 meters per second squared (m/s^2) towards the planet's center. This acceleration is commonly denoted as "1g" (one Earth gravity) (Nasa, 2023).

Microgravity is an exceptional milieu that elicits physical and physiological transformations in plants, necessitating a thorough comprehension of plant growth and advancement in microgravity for the realm of space agriculture. Owing to the limited availability of space travel, rotational contraptions like the clinostat (CL) and Radom Positioning Machine (RPM) have been employed to engender microgravity here on Earth. Genuine and simulated experiments conducted in microgravity advocate for a constructive influence of unilateral light exposure on the germination and maturation of seedlings, in contrast to etiolated seedlings (Villacampa et al., 2022).

Microgravity denotes a distinctive attribute of the space milieu, representing an altered gravitational condition that imposes abiotic stress on the metabolism, growth, and development of organisms. Diverse platforms, including the dropping tower at the Centre for Microgravity Research in Bremen, Germany, suborbital flights typically supported by national space agencies worldwide, and the clinostat, have been employed to simulate microgravity conditions. The International Space Station (ISS) has been an experimental platform to conduct research in true microgravity conditions. (Akomolafe *et al.*, 2017)

Microgravity is a state of weightlessness experienced in outer space due to very low gravitational pull, measured in μg , which is one millionth of Earth's gravity. In contrast, hyper gravity refers to conditions where gravity is greater than on Earth, measured in multiples of 1g, and can be artificially created using centrifuges or other means. (Oluwafemi and Ibraheem, 2021)

2. HISTORY

In 1946, the U.S. Army conducted the first experiments on the impact of microgravity on plants using captured V-2 rockets. These experiments were focused on studying the growth and development of plants in environments with reduced gravity. (Nasa, 2023) Early Space Experiments (1960s-1970s): Vostok, which refers to a series of manned Soviet spacecraft, initiated its first flight by sending the first human being into space. On April 12, 1961, Vostok 1, with cosmonaut Yury A. Gagarin on board, completed a single orbit around Earth before reentering the atmosphere. As part of the Soviet Union's Vostok 1 mission in 1961, Yuri Gagarin exposed wheat seeds to space conditions for a brief period in order to investigate their growth in microgravity. (Britannica, 2023) In 1982, the European Space Agency's Spacelab 1 mission provided a platform for conducting controlled experiments on plant growth in space. These experiments yielded valuable insights into the physiological and growth patterns of plants under the influence

of microgravity. (Nasa, 2023) The 1990s witnessed significant progress in microgravity plant research with the establishment of dedicated spaceflight facilities like NASA's Plant Growth Facility (PGF) and the European Modular Cultivation System (EMCS). These facilities enabled more controlled experiments and long-term studies on plant growth in space. In 2003, NASA's Expedition 6 crew successfully cultivated and consumed lettuce aboard the International Space Station (ISS). This achievement marked a significant milestone in achieving sustainable plant growth and food production in microgravity environments. NASA's Veggie Plant Growth System, deployed on the ISS in 2014, allows astronauts to cultivate various plants, including lettuce and zinnias, in order to further investigate the effects of microgravity on plant growth and explore the potential for fresh food production in space. (Nasa, 2023)

3. MICROGRAVITY SCIENCE

Microgravity science refers to the acquisition of knowledge or the study of matter, material, or a physical entity within a microgravity environment. This particular environment offers a distinct setting that allows for a comprehensive comprehension of the fundamental properties of matter. The absence of gravity often leads to unexpected behaviors and phenomena, which in turn can stimulate innovative ideas and advancements on Earth. It is important to note that engaging in experiments involving microgravity does not necessarily require space travel. There exist various methods to create temporary microgravity conditions here on Earth, such as employing free-fall conditions where gravity acts unopposed. This can be achieved through the utilization of massive drop towers, sounding rockets equipped with instruments, and parabolic aircrafts. (Rachel, 2018)

Generally, products manufactured within microgravity environments possess key attributes that often surpass those of their terrestrial counterparts. From a commercial standpoint, these products exhibit desirable features that facilitate effective marketing strategies. Moreover, the development of new plant varieties that are adapted to extreme conditions, as well as the production of superior goods, are made possible within this unique environment. Additionally, ensuring food security and accessibility to safe, nutritious, and culturally acceptable food at all times is vital for promoting healthy living. (Oluwafemi and Ibraheem, 2021)

The perfume industry behemoth, International Flavors and Fragrances (IFF), discovered an intriguing phenomenon in 1998 when they dispatched a miniature rose named "Overnight Scentsation" aboard the space shuttle Discovery (STS-95). This particular flower emitted a distinctive "floral rose aroma" that differed significantly from its usual fragrance on Earth. Subsequently, this novel scent was incorporated into "Zen," a perfume produced by the renowned Japanese company Shiseido. (Mamta, 2003)

4. HOW TO CREATE MICROGRAVITY CONDITION ON EARTH

The Clinostat is a device of elementary nature that is utilized in order to generate an artificial gravitational effect by manipulating the centrifugal force across a spectrum of angular velocities. As a clinostat with solely a singular axis of rotation, it operates in a two-dimensional (2-D) capacity, with said axis running perpendicular to the direction of the gravitational vector. Its functionality is contingent upon both the swiftness and orientation of its rotation. The act of rotating on a clinostat is referred to as "clinorotation" (United Nations, 2013). Furthermore, it is capable of providing rotational speeds ranging from 0 to 90 revolutions per minute (United Nations, 2013).

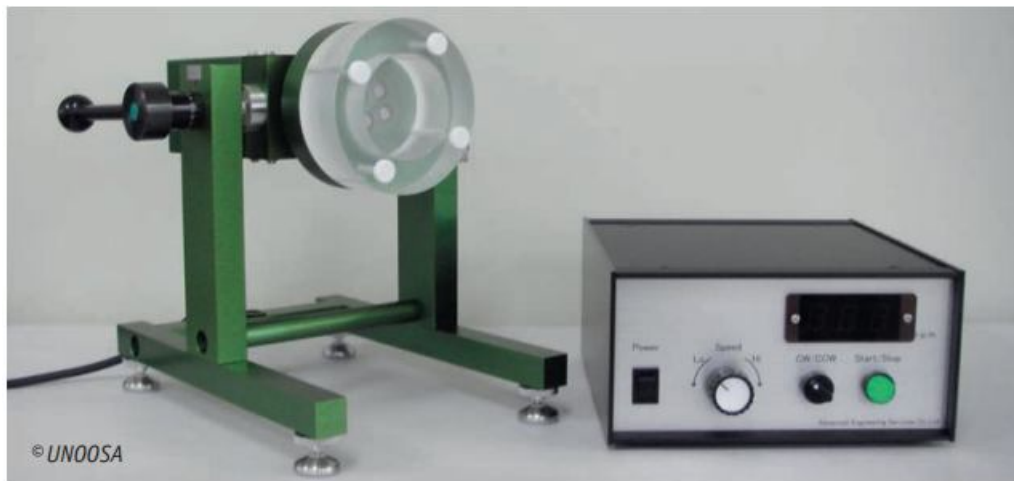


Fig 1. Picture of the one-axis clinostat

4.1 3-D CLINOSTAT

The 3D clinostat serves as a pivotal instrument in scientific inquiry, particularly within the realms of space biology, biomedical research, and plant physiology. Functioning as a simulated microgravity environment, this apparatus boasts a rotating platform with three pivotal axes—pitch, roll, and yaw. By effecting continuous changes in sample orientation with respect to gravitational forces through pitch axis movement, the 3D clinostat effectively negates the impact of gravity on biological specimens. This perpetual reorientation prevents cells or organisms from gravitational sensing, faithfully emulating the weightlessness encountered in space. With applications spanning cell growth, tissue development, and organism behavior, the 3D clinostat facilitates profound insights into the physiological adaptations of living entities to microgravity, holding profound implications for both space exploration and human health (Herranz et al., 2013)

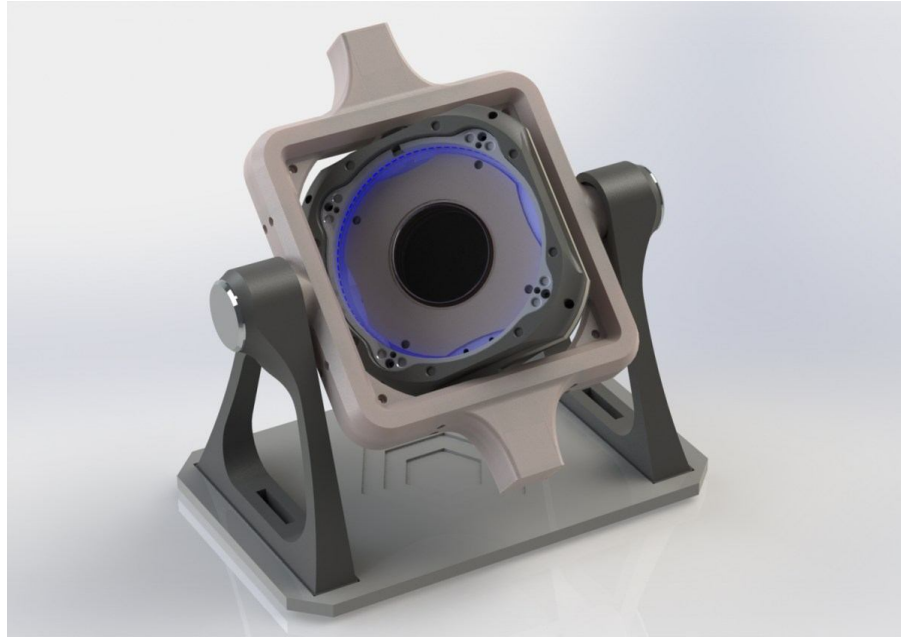


Fig 2: Picture of 3-d clinostat

5. IMPACT ON SEED GERMINATION & SEEDLING ESTABLISHMENT

5.1 Delayed germination:

The absence of gravity in microgravity conditions disrupts the typical settling and distribution patterns of water and nutrients, resulting in uneven moisture levels around seeds. This disparity complicates the absorption of water and essential nutrients crucial for germination, contributing to reduced oxygen levels compared to Earth. The diminished oxygen levels impede the respiratory process in seeds, which relies on oxygen for energy production, consequently decelerating metabolic processes and impeding germination (Ikhajagbe et al., 2021). Microgravity-induced challenges extend to reduced water uptake within the seed, posing obstacles to the embryo's ability to imbibe water and initiate germination. Furthermore, the absence of gravity in microgravity conditions adversely affects hormone signaling, a pivotal aspect of plant development regulation. This disruption in signaling pathways may result in delayed germination due to altered hormone regulation influenced by gravity. Additionally, microgravity interferes with nutrient uptake and distribution, potentially compromising the availability of essential nutrients essential for germination (Ikhajagbe et al., 2021).

Research findings pertaining to Zea mays demonstrate the impact of gravity on the final germination percentage and seedling dry weight. At 72 hours, the final germination

percentage was notably diminished, with rates of 60% at 2.0 rpm in the clinostat, 68% at 1.0 rpm, and 80% at 0.5 rpm, as opposed to the control group's 94%. A similar trend was observed in seedling dry weight, where a significant decrease correlated with an increase in clinostat rate, underscoring the influential role of gravity on these germination parameters (Ikhajagbe et al., 2021).

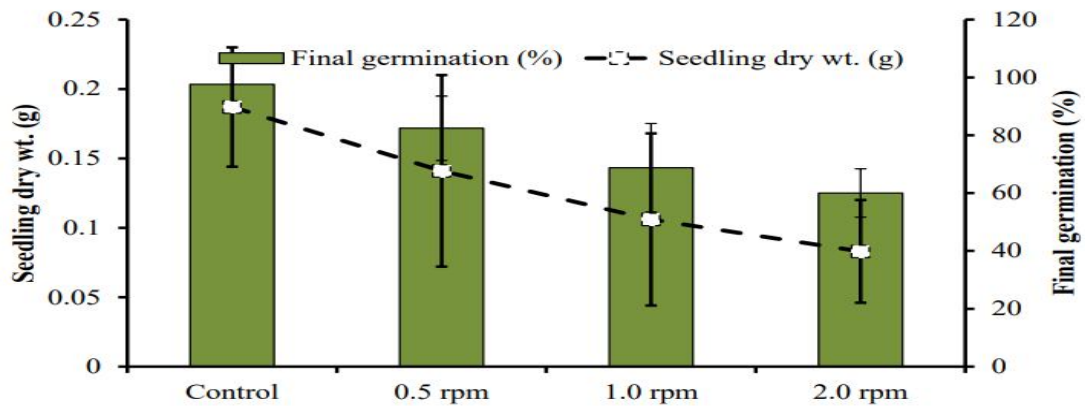


Fig 3: Effects of microgravity on the final germination percentage and seedling dry weight of *Zea mays* at 72 hrs after the initiation of germination

5.2 Altered growth orientation:

In conditions of microgravity, the absence of gravitational guidance induces aberrant growth patterns in plants, marked by haphazard orientations of roots, stems, and leaves, resulting in irregular and less predictable plant structures. The growth behavior of mung bean seedlings was investigated under controlled conditions and clinorotation. Notably, seedlings subjected to clinorotation exhibited a statistically significant increase in both fresh weight and water content compared to those grown under standard conditions. Furthermore, the clinorotated seedlings demonstrated a substantially elongated shoot and root in comparison to their counterparts under control conditions (Nakajima et al., 2021).

Table 1: Growth of mung bean seedlings under the control and clinorotation

Growth condition	Fresh weight (mg)	Water content (%)	Length (cm)	
			Stem	Root
Clinorotation	332.33 ± 8.53*	85.49 ± 0.44*	4.43 ± 0.21*	5.05 ± 0.36*
Control	298.67 ± 9.64	83.66 ± 0.45	3.35 ± 0.25	3.50 ± 0.31

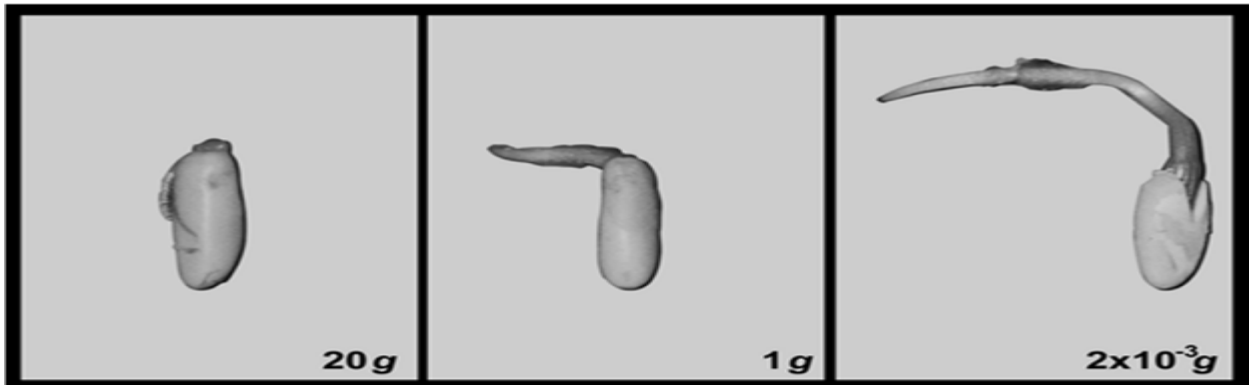
6. MICROGRAVITY INDUCED CHANGES IN MORPHOLOGY

6.1 Root Growth:

In the context of microgravity conditions, the process of root formation in plants undergoes significant alterations. Unlike under normal gravitational circumstances, roots in microgravity exhibit a stochastic growth pattern, deviating from the typical gravitropic response. The absence of gravitational cues impedes the establishment of root polarity and disrupts the usual elongation of roots. Consequently, the dearth of gravity-induced signals gives rise to an augmented formation of lateral roots, which are smaller offshoots branching from the primary root. This phenomenon results in the development of a more compact and efficacious root system, facilitating enhanced absorption of nutrients and water (Manzano et al., 2018).

Furthermore, observations pertaining to the morphological aspects of germinating seeds corroborate findings derived from enzymatic activity assays conducted under both microgravity and hypergravity conditions. Seeds cultivated in simulated microgravity and hypergravity conditions exhibit root lengths of approximately 2 ± 0.32 cm and 0.1 ± 0.02 cm, respectively, contrasting with those cultivated at 1 g, where the root length averages around 0.6 ± 0.13 cm (Faraoni et al., 2019).

Fig 4: Pinus pinea seed germination (14th day) at different simulated gravity condition.



6.2 Stem elongation:

In the absence of gravitational forces, plants exhibit a phenomenon termed "spaceflight-associated plant growth" (SAPG), marked by heightened stem elongation. Etiolated seedlings, when subjected to darkness, manifest a notable increase in shoot length compared to counterparts exposed to alternative light conditions. The activation of a developmental program known as skotomorphogenesis ensues in shoot-root communication alterations (Villacampa, A et al., 2022).

Observations and recordings of root and plumule growth were conducted. The plumule length of seedlings positioned on a bench surface without clinorotation measured 1.3 cm, exhibiting no statistically significant difference ($P > 0.05$) in comparison to seedlings subjected to clinorotation. The presented figures illustrate radicle and plumule emergence and elongation in clinorotated seeds, which displayed greater lengths in both plumule and radicle in contrast to figures representing seed growth under the influence of gravitational forces over the same duration.

Table 2 Measurements taken between plant under the influence of gravity and microgravity

	Plumule length (cm)	Radicle length (cm)
Flat surface	1.3 ^a	1.5 ^a
90 turned	1.6 ^a	2.1 ^a
3 rpm	1.8 ^a	5.6 ^a
2 rpm	2.1 ^a	6.3 ^a
1 rpm	1.3 ^a	4.5 ^{ab}

p-value	0.535	0.0127
---------	-------	--------



Fig.no.5 Growth of a clinorotated seedlings of maize



Fig.no.6 Growth of seedlings of maize under gravity

7. PHYSIOLOGICAL AND BIOCHEMICAL RESPONSE To MICROGRAVITY

7.1 Influence phytochrome system:

The absence of gravitational forces in space induces alterations in the spatial arrangement and orientation of phytochrome proteins within plant cells. These changes impede the customary signaling pathways regulated by phytochromes, thereby impacting fundamental physiological processes such as seed germination, stem elongation, and flowering. Discrepancies in the temporal patterns and duration of light exposure further disturb the typical photoperiodic responses of plants. This disruption is attributed to a biological light switch encompassing photoreceptors that monitor alterations in light intensity, duration, and color. These photoreceptors bind with tetrapyrrole chromophores and exist in two interconvertible forms, namely Pr and Pfr. Notably, certain flowering plants maintain a red-light sensory system to facilitate phototropism even in microgravity conditions.

7.2 Gas exchange & photosynthesis:

In the absence of buoyancy-driven convective flows, gases exhibit enhanced diffusion efficiency. Microgravity conditions are postulated to induce distinct distribution patterns of chloroplasts, the cellular organelles responsible for photosynthesis, within

plant cells. This potential alteration holds promise for optimizing gas exchange between plant tissues and their immediate surroundings, thereby facilitating increased assimilation of carbon dioxide (CO₂) essential for photosynthesis and improved elimination of oxygen (O₂) generated during the photosynthetic process. Such modified chloroplast distribution has the potential to enhance light capture and consequently augment the overall efficiency of photosynthesis. Moreover, the impact of reduced gravity on stomatal conductance contributes to alterations in gas exchange dynamics.

According to Paul and Ferl (2016), significant changes in total chlorophyll (Chl) content occur under altered gravity conditions, particularly in the Clino 2 treatment, exhibiting a notable 58.7% enhancement compared to the control. The Clino 2 treatment further manifests significant modifications in Chl a and b contents, registering increases of 73.5% and 42.4%, respectively, compared to the control. Importantly, the elevated level of Chl a surpasses that of Chl b, thereby influencing the Chl a/b ratio, which undergoes a noteworthy 22.2% and 21.6% enhancement under Clino 2 compared to the control (Hassanpour and Abdel Latef, 2023).

Table 3 Impact of altered gravity on chlorophyll (Chl) biosynthesis of *O. basilicum* seedling

Parameters	Gravity condition		
	Con (1 g)	Clino 3(0.013 g)	Clino 2 (0.009 g)
Chla($\mu\text{g g}^{-1}$ FW)	61.28 \pm 5.33 ^b	63.76 \pm 3.56 ^b	106.32 \pm 8.67 ^a
Chlb ($\mu\text{g g}^{-1}$ FW)	54.99 \pm 4.52 ^b	58.35 \pm 6.94 ^b	78.29 \pm 5.39 ^a
Total Chl($\mu\text{g g}^{-1}$ FW)	116.27 \pm 14.23 ^b	122.11 \pm 15.56 ^b	184.61 \pm 19.71 ^a
Chla/b	1.11 \pm 0.14 ^b	1.09 \pm 0.09 ^b	1.35 \pm 0.18 ^a

The catalytic efficiency of Rubisco exhibited a notable correlation with growth parameters in response to modified gravitational conditions. Particularly, in seedlings subjected to Clino 3 and 2 treatments, Rubisco activity displayed a substantial augmentation, registering a 32.83% and 43.28% increase compared to the control group, as reported by Hassanpour and Abdel Latef (2023)

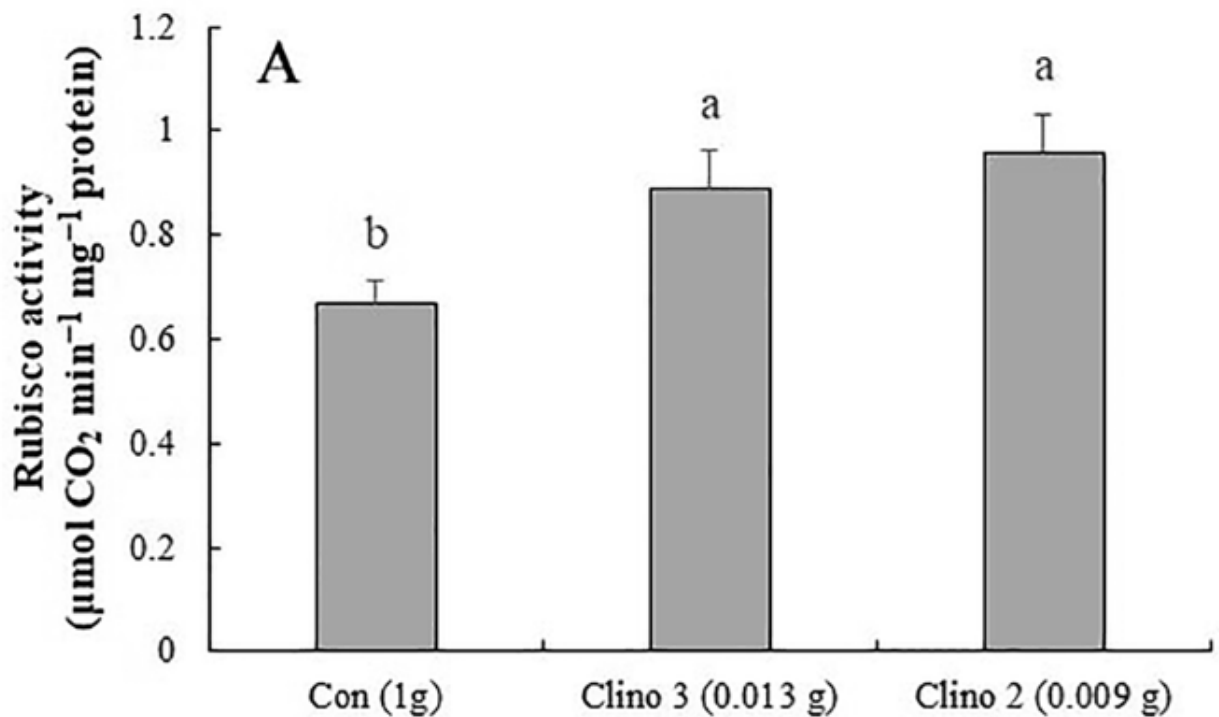


Fig.7: Rubisco enzyme activity

7.3 Secondary metabolites: Secondary metabolites, chemical compounds synthesized by plants not directly involved in growth or development, play pivotal roles in defense against pathogens, attraction of pollinators, and other ecological functions. Research indicates that exposure to microgravity can influence the biosynthesis and accumulation of secondary metabolites in plants, resulting in alterations in composition with potential implications for human health (Agarwal et al., 2018).

Plants cultivated in microgravity environments exhibit heightened production of secondary metabolites in comparison to their terrestrial counterparts. This augmented production, encompassing compounds such as flavonoids, phenolics, and alkaloids, is believed to be a response to the perceived stress of microgravity, serving as a defense mechanism against various environmental challenges, including herbivores and pathogens (Hassanpour and Abdel Latef, 2023).

The antioxidant properties of *O. basilicum* seedlings were examined, revealing notable increases in phenolic and flavonoid compounds under altered gravity conditions. Among these conditions, the Clino 2 treatment demonstrated the highest content of these compounds. Furthermore, anthocyanin content exhibited a twofold increase in seedlings subjected to the Clino 2 treatment compared to the control. Carotenoid content also

experienced a significant rise during clinorotation, reaching its peak at Clino 3 with approximately 26.27% higher content than the control (Hassanpour and Abdel Latef, 2023).

Table 4 Impact of altered gravity on the accumulation of antioxidant compounds in *O.basilicum* seedlings

Antioxidant compounds	Gravity conditions		
	Con (1 g)	Clino 3 (0.013 g)	Clino 2 (0.009 g)
Phenolics (mg g ⁻¹ DW)	0.73 ± 0.045 ^b	0.91 ± 0.069 ^a	1.02 ± 0.077 ^a
Flavonoids (mg g ⁻¹ DW)	0.32 ± 0.22 ^b	0.38 ± 0.069 ^b	0.79 ± 0.045 ^a
Anthocyanin (μmol g ⁻¹ FW)	254.25 ± 17.6 ^b	269.95 ± 31.2 ^b	519.7 ± 42.4 ^a
Carotenoids(μg g ⁻¹ FW)	15.53 ± 0.88 ^b	19.61 ± 1.25 ^a	17.03 ± 0.77 ^{ab}

8. MECHANO- CELLULAR RESPONSE TO MICROGRAVITY

8.1 Disruption of cytoplasmic streaming:

Cytoplasmic streaming, a phenomenon observed in plant cells, encompasses the dynamic movement of cytoplasm, contributing significantly to diverse cellular processes such as nutrient transport, organelle distribution, and intracellular signaling. The pivotal role of tubulin in the genesis of microtubules is indispensable for both cytoplasmic streaming and cellular division, as elucidated by Hoson et al. (2014).

8.2 Rigidity of cell wall:

The heightened gravitational conditions, as observed in hypergravity, lead to an augmentation in cell wall rigidity attributable to the accumulation of polysaccharides. This accrual is a consequence of the restrained degradation subsequent to polymerization. Conversely, under conditions of microgravity, the converse effect is observed, wherein the diminished gravitational forces result in a reduction of cell wall rigidity due to the

suppressed breakdown following polymerization, as elucidated by Wakabayashi et al. (2009).

8.3 Damage Severity of Cell Membranes:

Exposure to microgravity has been observed to induce oxidative stress in cellular structures, leading to the potential compromise of cell membrane integrity. This compromise results in a loss of selective permeability, leading to the extrusion of intracellular electrolytes, including salts and organic acids. Submersion of plant materials in distilled water exacerbates this phenomenon, causing an increase in the electrical conductivity (EC) of the distilled water due to the exosmosis of electrolytes.

The severity of cellular damage is directly proportional to the elevated EC values, and the extent of damage can be quantified by determining the relative ratio of EC values between damaged and undamaged tissues. A study conducted by Xu et al. in 2014 investigated the EC of tissue exudates from both 1g control and simulated-microgravity (RPM-treated) conditions at various time points (10, 20, and 30 days after planting, DAP). The analysis revealed a progressive exacerbation of membrane damage over time under simulated-microgravity conditions.

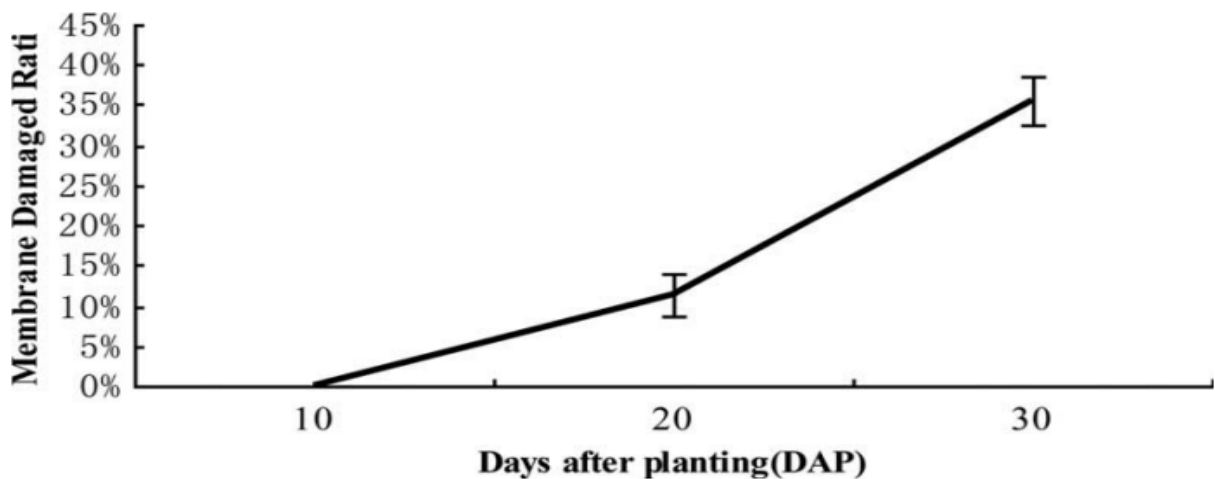


Fig 8: Cell membrane damage ratio of tomato seedlings grown in simulated microgravity

8.4 Cell wall extensibility:

Microgravity has been observed to induce heightened cell wall extensibility in specific plant species, potentially attributable to alterations in the functionality of proteins present in cell membranes, including ion channels and transporters. The absence of gravitational mechanical stresses on plant cell walls may contribute to increased flexibility and extensibility. Hoson et al. (2013) have suggested that modifications in the operation of these proteins can lead to disturbances in membrane integrity and function. Notably, the mechanical extensibility of the cell wall exhibited a notable elevation in the apical

elongating region of inflorescences, with a marked decline towards the basal region. Moreover, under microgravity conditions, cell wall extensibility in the apical and subapical regions was significantly higher compared to ground and on-orbit controls, implying potential positive implications for plant growth in a microgravity environment.

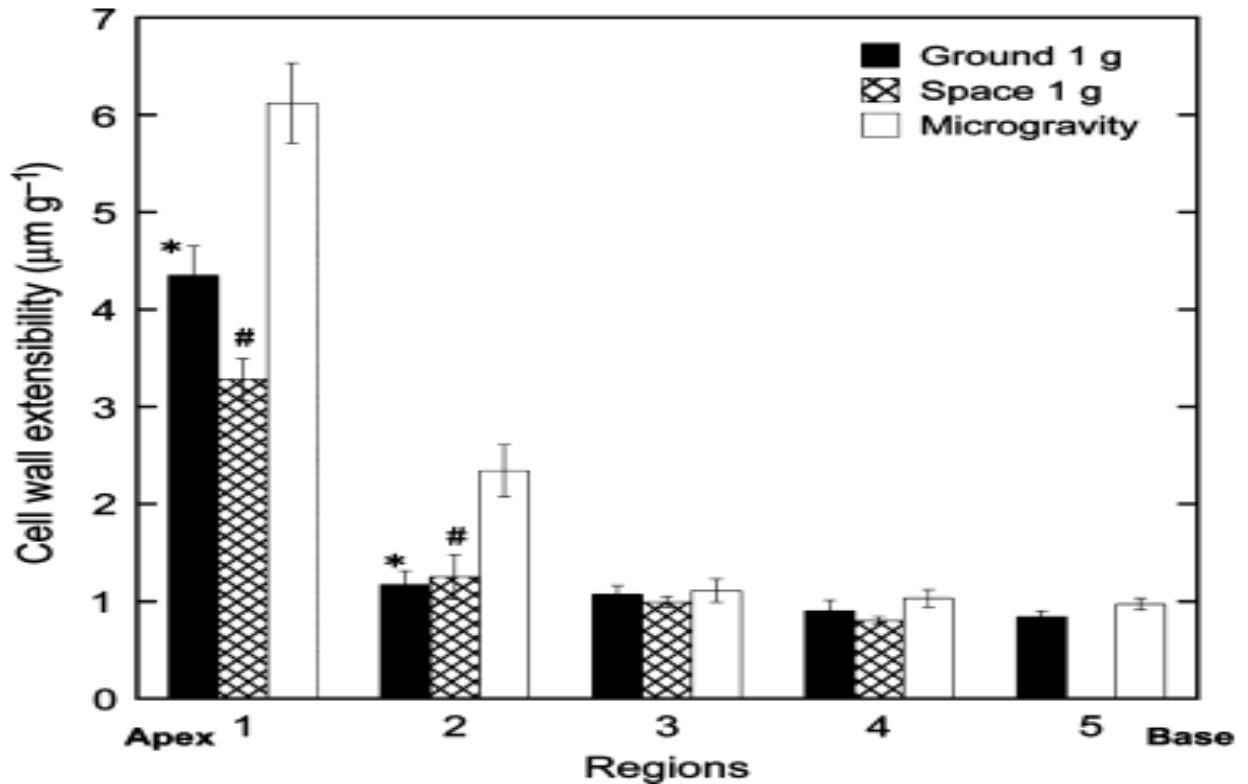


Fig 9: The cell wall extensibility along inflorescence regions

9. IMPACT ON PLANT LIFE CYCLE, FLOWERING AND SEED DEVELOPMENT

9.1 Delayed flowering:

Microgravity induces disruptions in signaling pathways crucial for the regulation of flowering time, notably affecting the photoperiod and gibberellin pathways. The absence of gravitational cues is postulated to perturb the internal circadian clock of plants, resulting in a delayed onset of flowering. Plants, dependent on photoreceptors for light signal perception, exhibit altered responses to photoperiods due to variations in light distribution in microgravity compared to Earth. Consequently, plants may flower at disparate times or experience delays in the flowering process (Xie and Zheng, 2020).

To elucidate the impact of microgravity on plant flowering, *Arabidopsis* seedlings at the 9-day-old stage were cultivated on a 3-D clinostat under long-day (LD) photoperiod conditions for 6 days. Subsequently, these seedlings were transferred to a greenhouse under 1 g stationary control conditions for a period of 2 weeks. *Arabidopsis* plants subjected to the 1 g control conditions exhibited flowering approximately 22 days after

sowing. In contrast, those exposed to the rotational conditions of the 3-D clinostat displayed a notable delay, with flowering occurring approximately 1 week later than the control plants. Furthermore, Arabidopsis plants in the control conditions reached the flowering stage with around 8 rosette leaves, while those in the 3-D clinorotated conditions flowered after the emergence of 11 rosette leaves. These findings strongly imply that 3-D clinorotation has the potential to induce a delay in the flowering process of Arabidopsis (Xie and Zheng, 2020).

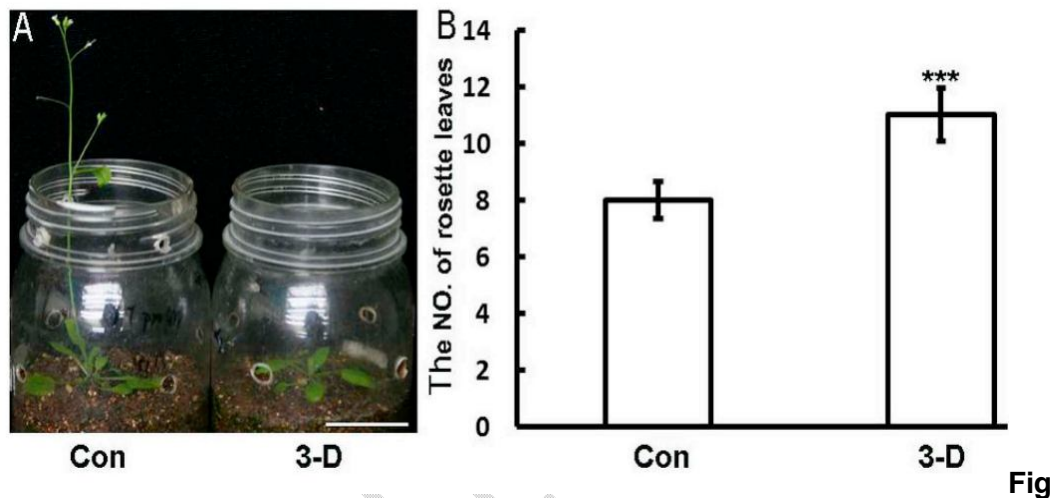


fig10:3-D clinorotation delayed Arabidopsis flowering

10. IMPACT ON PLANT ANATOMY

10.1 Vascular development of cotyledons:

The vascular structures in both clinorotated seedlings and the control group exhibited vessels characterized by spiral thickening. Lignification proceeded normally in both instances, commencing at cell corners in the middle lamella primary wall and subsequently extending throughout the entire secondary wall towards the cell lumen, as outlined by De Micco et al. (2006).

The average count of principal veins was 14.2 in clinorotated seedlings, slightly surpassing the count of 13.2 in the control group, although this discrepancy lacked statistical significance. Notably, a statistically significant disparity was observed in the number of vessels per vein, with clinorotated seedlings showing a higher mean count of 9.37 vessels per vein (SE = 0.53) compared to the control group's mean count of 7.57 vessels per vein (SE = 0.42), as reported by Oluwafemi and Ibraheem (2021)

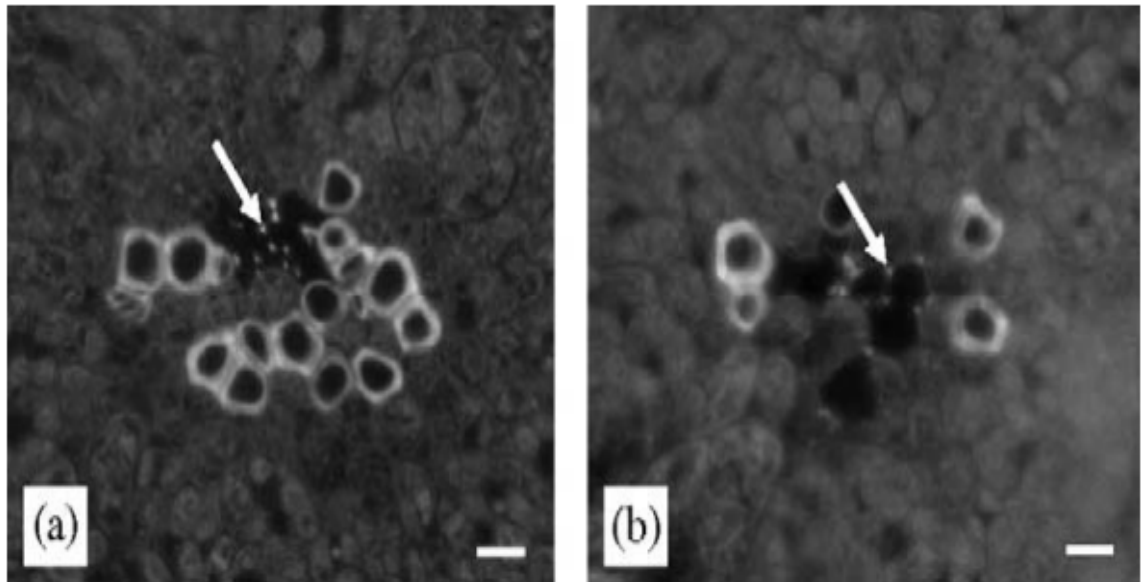


Fig 11: Epifluorescence microscopy views of thin sections of cotyledons showing vascular development of clinorotated (a) and control (b) seedlings. Arrows point to lignification starting at cell corners.

SPACE FARMING

Space farming involves the cultivation of food in extraterrestrial environments, such as space stations or celestial bodies beyond Earth. This practice contributes to the advancement of agricultural knowledge on Earth, as insights gained from growing crops in the inhospitable conditions of space can be applied to similarly challenging climates on our planet. Astrobotany, the study of plants in space, focuses on understanding how microgravity and space radiation impact the germination, growth, and development of plants. Additionally, investigating the influence of gravity, light, atmospheric conditions, and other factors on a plant's ability to thrive in space is crucial.

Similar to Earth, the fundamental requirements for planting seeds in space include nutrients, oxygen, water, and sufficient light, whether in soil or soil-less cultivation systems. Given the adverse environmental conditions in space, closed, controlled, or soil-less cultivation methods are employed. Essential components of space farming include soil simulation, soil-less cultivation, in-soil cultivation, burying habitats, and genetic engineering of crops.

Various methods are employed for planting crops in space, such as plant pillows, plant agar, greenhouses, aeroponics, hydroponics, and aquaponics (Oluwafemi and Oluwafemi, 2017).

RELEVANCE TO CROP GROWTH IN MICROGRAVITY

Developing efficacious strategies for the preservation of seeds and ensuring their

viability during prolonged periods in space stations and exploration initiatives is paramount for the sustainable cultivation of food and agriculture in extraterrestrial environments. The establishment of plant-centric life support systems, characterized by sustainability and the ability to sustain astronauts over extended durations in space stations and protracted space missions, assumes pivotal significance. This imperative arises from the necessity to diminish reliance on Earth-bound resupply missions and safeguard the overall well-being of astronauts.

The identification of attributes conducive to successful plant growth under the demanding conditions of deep space exploration constitutes a critical pursuit. This imperative is essential to secure the enduring sustainability of forthcoming space missions and the creation of habitats in extraterrestrial landscapes. These attributes encompass a diverse array of characteristics that empower plants to thrive in the unique milieu of deep space. Notably, adaptability to extreme conditions, such as heightened radiation levels, microgravity, and limited nutrient availability, emerges as a crucial set of traits. Plants exhibiting efficiency in utilizing minimal resources, specifically water and nutrients, while concurrently maintaining robust growth, are indispensable. Additionally, traits related to stress tolerance, encompassing resistance to temperature fluctuations and adaptability to variable light intensities, assume vital significance for plant survival in the space environment.

Enhancing agricultural practices on Earth, particularly in regions marked by harsh and extreme conditions, assumes pivotal importance for ensuring food security and sustainable development in locales where conventional farming encounters substantial challenges. Such regions encompass arid deserts, high-altitude mountainous terrains, polar landscapes, and areas characterized by unpredictable weather patterns.

A pivotal objective for future space exploration initiatives revolves around reducing dependence on freeze-dried foods for astronauts. While freeze-dried foods have historically served as a reliable source of nutrition for astronauts, diversifying the space diet presents numerous advantages. Firstly, it mitigates issues of monotony and potential psychological challenges associated with prolonged consumption of homogeneous food varieties during extended missions. A diversified menu is anticipated to substantially elevate morale and contribute to the mental well-being of astronauts.

Secondly, the inclusion of fresh foods offers essential vitamins and nutrients that may undergo degradation during the freeze-drying process. The incorporation of fresh produce, including fruits and vegetables, is anticipated to contribute to a more wholesome and balanced diet, thereby supporting the physical health of astronauts and mitigating the risk of diet-related health complications.

CONSTRAINTS

Managing the distribution of water, fluctuations in temperature, variations in atmospheric pressure, and exposure to radiation are considerable challenges encountered in the low-gravity environments of space, wherein astronauts and plant life coexist. The absence of gravitational force renders water distribution a complex

undertaking. In conditions of microgravity, water does not flow naturally, necessitating meticulous engineering to ensure that plants receive adequate hydration. Sophisticated systems such as capillary-based fluidics and precise irrigation mechanisms are imperative in regulating moisture levels surrounding the roots of plants.

Significant apprehension arises from the occurrence of extreme temperature fluctuations. In space, temperatures can fluctuate considerably from scorching heat when exposed to direct sunlight, to frigid cold when in the shadow of spacecraft. These fluctuations can disrupt the physiological and biochemical processes of plants, thereby affecting their growth and productivity. The implementation of insulation and shading systems, in conjunction with temperature-regulated growth chambers, aids in mitigating these fluctuations.

Another challenge is presented by the variable pressure in space resulting from the absence of a natural atmosphere. Plants have evolved to thrive within specific pressure ranges on Earth. In the vacuum of space, the maintenance of suitable pressures is crucial for the well-being of plants. Controlled environments and chambers regulated for pressure are indispensable in creating stable conditions for plant growth.

The heightened radiation levels in space are a significant concern for both astronauts and plants. High-energy particles emitted by the sun and cosmic rays have the potential to damage plant DNA, leading to mutations, autoimmune disorders, and irregular growth patterns. The utilization of shielding materials and specialized growth chambers equipped with radiation protection serves to diminish the detrimental effects of space radiation on plants. Furthermore, the exploration selecting or genetically modifying plants to possess enhanced radiation tolerance is a viable avenue of investigation.

Discussion

1. Role of major hormone in delayed germination under microgravity

Hormones, such as auxins and gibberellins, play crucial roles in regulating germination. These hormones are involved in processes like cell elongation, seed dormancy, and the breaking of dormancy during germination. In microgravity environments, the absence of a clear gravitational signal might affect the normal hormonal regulation in plants.

Previous experiments in space, such as those conducted on the International Space Station (ISS), have shown that plants may exhibit altered growth patterns, including changes in root orientation and overall plant architecture. It's possible that the absence of a strong gravitational signal in microgravity could lead to altered hormonal responses, potentially resulting in delayed germination. Absence or reduction of gravity might affect the distribution of hormones within the plant and interfere with their normal signaling pathways.

2. Oxidative stress

ROS, including molecules like superoxide radicals, hydrogen peroxide, and singlet oxygen, are natural byproducts of plant cellular processes. Under stress conditions, including microgravity, there might be an increase in ROS production. Plants have

developed sophisticated antioxidant defense mechanisms to counteract the harmful effects of ROS. These mechanisms include enzymes like superoxide dismutase, catalase, and peroxidase, as well as non-enzymatic antioxidants.

3. Importance of BIO-KES

Ethylene is a natural hormone that causes plant spoilage and premature withering if present in excess amounts. Produced by plants, it acts as a chemical cue that tells plants to begin ripening. It is critical to remove ethylene from enclosed plant-growth environments, such as those for growing in space, because high concentration levels can be detrimental to the plants. WCSAR, in conjunction with Anderson's technology, created an ethylene scrubber for plant growth chambers. This innovation presents commercial benefits for the food industry in the form of a new device, named Bio-KES. Incorporating the WCSAR filter system, Bio-KES removes ethylene and helps to prevent spoilage. The system's fan draws in air and passes it over pellets treated with titanium dioxide. The titanium dioxide works as a catalyst to break down the ethylene into carbon dioxide and water vapor. This change is triggered by photocatalysis, a process that uses ultraviolet light to activate the titanium particles. The by-products of carbon dioxide and water vapor are then recirculated back into the storage or display area.

The unique process of the Bio-KES systems has advantages over other ethylene removal systems. Most systems simply oxidize the ethylene in the air with an oxidant such as potassium permanganate, and consequently, require frequent maintenance to remove exhausted oxidant pellets. Bio-KES breaks down ethylene catalytically, thereby eliminating build-up, so the system is almost maintenance-free. Because air continuously passes through the device, Bio-KES removes approximately 99 percent of the present ethylene and concentrations are unable to reach harmful levels.

4. Role of microgravity in vascular development

Plants grown in microgravity often exhibit changes in overall morphology, including alterations in root and shoot growth. The vascular system is essential for the transport of water, minerals, and nutrients between these plant parts. Microgravity conditions can affect the orientation and elongation of plant roots. Changes in root growth can influence the development of lateral roots and their connection to the vascular system. Microgravity can impact the differentiation and maturation of xylem and phloem tissues. This can influence the efficiency of water and nutrient transport within the plant.

5. Effect of microgravity experiment conducted on space stations

Experiments conducted in microgravity environments, such as those aboard the International Space Station (ISS) or during parabolic flights, provide valuable insights into how various biological, physical, and chemical processes behave in the absence of gravitational forces or under altered gravitational conditions.

Plants grown in microgravity often exhibit changes in growth patterns, including alterations in root and shoot architecture. The absence of a clear gravitational signal can affect

tropisms and orientation responses. Fluid behavior is different in microgravity due to the absence of buoyancy-driven convection. This impacts the dynamics of fluid movement, such as capillary flow, which has implications for nutrient transport in plants and fluid-based experiments. Microgravity can influence cell structure and morphology. Cells may show differences in shape, size, and arrangement compared to cells grown in a gravitational field.

6. How can we set microgravity level on clinostat

A clinostat is a laboratory apparatus used to simulate microgravity conditions on Earth. It typically consists of a rotating device that continuously reorients samples, preventing the perception of a specific gravitational direction.

Securely mount the biological or physical sample you want to subject to simulated microgravity on the clinostat. This can include plant seeds, cell cultures, or other experimental materials. Clinostats operate by rotating the sample around an axis, preventing the perception of a specific gravitational direction. Adjust the rotation speed of the clinostat based on the specific experimental conditions you want to simulate. The rotation speed is crucial as it influences the effective gravity (g) that the samples experience. Regularly monitor and calibrate the clinostat to ensure that it is operating correctly. Calibration involves confirming that the rotation speed is accurate and that the samples experience the desired conditions. Determine the duration of exposure needed for your experiment. Some studies may require continuous exposure to simulated microgravity, while others may involve intermittent exposure. Depending on your experimental goals, you may need to consider additional parameters such as temperature, humidity, and lighting conditions. Ensure that these factors are controlled and maintained throughout the experiment. Record observations and measurements during and after exposure to simulated microgravity. Analyze the results to understand the effects of microgravity on your experimental system.

CONCLUSION

The lack of gravitational impact can have an impact on the alignment of roots and shoots, rendering the process of seed establishment more challenging. Over prolonged durations in the absence of gravity, certain flora species possess the capability to acclimate and evolve mechanisms to confront these formidable circumstances. Analyzing these accommodations can provide valuable insights for forthcoming extraterrestrial agricultural undertakings. Elucidating the fundamental comprehension of plants or plant cells in relation to the perception, stimulus, and response or adjustment to the microgravity environment is of utmost importance.

FUTURE ASPECTS

Screening and identifying the most suitable plant/crop candidates for space agriculture is an essential step in the establishment of sustainable food production systems for future space exploration and colonization. These candidates should possess characteristics such as adaptability to extreme environmental conditions, a high nutritional value, and efficient resource utilization.

Once appropriate plants are chosen, it becomes imperative to elucidate the cellular and molecular mechanisms that govern their responses to the entirety of space environmental factors, rather than solely microgravity. This comprehensive understanding is crucial due to the diverse stressors present in space environments, including radiation, fluctuations in temperature, altered atmospheric pressure, and limited resources. Researchers must investigate how these factors impact plant growth, development, and metabolism at the cellular and molecular levels. This knowledge can then be utilized to inform genetic modification or selective breeding efforts aimed at enhancing a plant's resilience and productivity in space.

Extensive studies on plant-microbial interactions are equally indispensable. Microbes play a pivotal role in nutrient cycling, disease suppression, and soil health. In space environments where sterile conditions are prevalent, maintaining these interactions becomes challenging yet essential. Research should focus on the development of closed-loop systems that incorporate beneficial microbes to support plant growth and nutrient cycling, thus contributing to a more sustainable space agriculture ecosystem.

Moreover, it is necessary to develop specialized plant varieties through breeding programs or genetic engineering. These varieties should demonstrate prolific growth and high yields under protected cultivation systems specifically designed for the adverse conditions of celestial bodies lacking a natural atmosphere. Traits such as radiation resistance, efficient water and nutrient utilization, and tolerance to extreme temperatures become paramount in such environments.

REFERENCES

- NASA [National Aeronautics and Space Administration]. 2023. Science that can't be done on earth [on-line]. <https://www.nasa.gov/> [10 Sept 2023].
- Akomolafe, G.F., Omojola, J., Joshua, E.S., Adediwura, S.C., Adesuji, E.T., Odey, M.O., Dedeke, O.A. and Labulo, A.H., 2017. Growth and Anatomical Responses of *Lycopersicon esculentum* (Tomatoes) under Microgravity and Normal Gravity Conditions. *Int. J. of Bioengineering and Life Sciences*, 11(5): 367-370.
- De Micco, V., Aronne, G. and De Pascale, S. 2006. Effect of simulated microgravity on seedling development and vascular differentiation of soy. *Acta Astronautica*, 58(3) : 139-148.
- Ferl, R.J. and Paul, A.L.kya. 2016. The effect of spaceflight on the gravity-sensing auxin gradient of roots: GFP reporter gene microscopy on orbit. *npj Microgravity*. 2(1): 1-9.
- Hassanpour, H. and Latef, A.A.H. 2023. Changes in chlorophyll metabolism, Rubisco gene expression, and antioxidant metabolites of *Ocimum basilicum* seedlings subjected to clinorotation. *Plant Cell, Tissue and Organ Cult. (PCTOC)*. 153(1): 119-132.
- Ikhajagbe, B., Anoliefo, G.O., Orukpe, A.O. and Ibrahim, M.S. 2023. Effects of selected plant growth stimulators on enhancing germinability and germination parameters of *Zea mays* L. under microgravity conditions simulated by a two-dimensional clinostat. *Int. J. of Horti. Sci.* 10(1): 1-10.
- Hoson, T., Soga, K., Wakabayashi, K., Hashimoto, T., Karahara, I., Yano, S., Tanigaki, F., Shimazu, T., Kasahara, H., Masuda, D. and Kamisaka, S. 2014. Growth stimulation in inflorescences of an Arabidopsis tubulin mutant under microgravity conditions in space. *Plant Biology*, 16: 91-96.
- Iovane, M., Izzo, L.G., Romano, L.E. and Aronne, G., 2023. Simulated microgravity affects directional growth of pollen tubes in candidate space crops. *Frontiers in Plant Science*, 14 : 1186- 1196.
- Musgrave, M.E., Kuang, A. and Matthews, S.W. 1997. Plant reproduction during spaceflight: importance of the gaseous environment. *Planta*, 203: 177-S184.

- Nakajima, S., Nagata, M. and Ikehata, A. 2021. Mechanism for enhancing the growth of mung bean seedlings under simulated microgravity. *npj Microgravity*, 7(1): 26.
- Office for Outer Space Affairs. 2013. Teacher's Guide to Plant Experiments in Microgravity. Human Space Technology Initiative. United Nations, 56p. Available: [ZGIP \(unoosa.org\)](https://www.unoosa.org/). [11 Sept 2023].
- Oluwafemi, F.A., (2017). Microgravity research and applications: Roles in economic prosperity and poverty reduction. In: *Conference proceedings of United Nations/United Arab Emirates high level forum*; 7-9 Sept, 2021. United Nations Office for Space Affairs, Dubai, UAE [on-line]. Available: <https://www.unoosa.org/documents/pdf/psa/activities/2021/UNAustria2021/DAY2/OLUWAFEMI.pdf> [10 Sept 2023].
- Porterfield, D.M., 2002. The biophysical limitations in physiological transport and exchange in plants grown in microgravity. *J. of Plant Growth Regulation*. 21(2).
- Rachel Brazil (2018). Science in microgravity [on-line]. Available: <https://www.chemistryworld.com/features/science-in-microgravity/3009826.article> [10 Sept 2023].
- Villacampa, A., Fañanás-Pueyo, I., Medina, F.J. and Ciska, M. 2022. Root growth direction in simulated microgravity is modulated by a light avoidance mechanism mediated by flavonols. *Physiologia Plantarum*. 174(3): 1372-1385.
- Xu, D., Guo, S. and Liu, M. 2014. Effects of long-term simulated microgravity on tomato seedlings. *Canadian J. of Plant Sci.* 94(2): 273-280.
- Wakabayashi, K., Nakano, S., Soga, K. and Hoson, T. 2009. Cell wall-bound peroxidase activity and lignin formation in azuki bean epicotyls grown under hypergravity conditions. *J. of Plant Physio.*166(9): 947-954.
- Xie, J. and Zheng, H. 2020. Arabidopsis flowering induced by photoperiod under 3-D clinostat rotational simulated microgravity. *Acta Astronautica*, 166 :567-572.
- Paul, A.-L., & Ferl, R. J. (2018). Plant biology in reduced gravity on the Moon and Mars. *Plant Science*. 276: 85-94.
- Agarwal, A., Joshi, R. and Agarwal, S. 2018. Impact of microgravity on secondary metabolism in plants. *Acta Astronaut.* 153: 278-284.
- Faraoni, P., Sereni, E., Gnerucci, A., Cialdai, F., Monici, M. and Ranaldi, F. 2019. Glyoxylate cycle activity in Pinus pinea seeds during germination in altered gravity conditions. *Plant Physio and Biochem*, 139: 389-394.
- Herranz, R., Anken, R., Boonstra, J., Braun, M., Christianen, P. C., de Geest, M., and Medina, F. J. (2013). Ground-based facilities for simulation of microgravity:

- organism-specific recommendations for their use, and recommended terminology. *Astrobiology*, 13(1), 1-17.
- NASA [National Aeronautics and Space Administration]. 2023. Science that can't be done on earth [on-line]. <https://www.nasa.gov/> [10 Sept 2023].
- Ferl, R.J. and Paul, A.L. 2016. The effect of spaceflight on the gravity-sensing auxin gradient of roots: GFP reporter gene microscopy on orbit. *npj Microgravity*. 2(1): 1-9.
- Hassanpour, H. and Latef, A.A.H. 2023. Changes in chlorophyll metabolism, Rubisco gene expression, and antioxidant metabolites of *Ocimum basilicum* seedlings subjected to clinorotation. *Plant Cell, Tissue and Organ Cult. (PCTOC)*. 153(1): 119-132.
- Ikhajiagbe, B., Anoliefo, G.O., Orukpe, A.O. and Ibrahim, M.S. 2023. Effects of selected plant growth stimulators on enhancing germinability and germination parameters of *Zea mays* L. under microgravity conditions simulated by a two-dimensional clinostat. *Int. J. of Horti. Sci.* 10(1): 1-10.
- Villacampa, A., Fañanás-Pueyo, I., Medina, F.J. and Ciska, M. 2022. Root growth direction in simulated microgravity is modulated by a light avoidance mechanism mediated by flavonols. *Physiologia Plantarum*. 174(3): 1372-1385.
- Xu, D., Guo, S. and Liu, M. 2014. Effects of long-term simulated microgravity on tomato seedlings. *Canadian J. of Plant Sci.* 94(2) : 1- 15.