International Journal of Emerging Research in Science, Engineering, and Management
Vol. 1, Issue 1, pp.10-14, July 2025.
www.ijersem.com

Effect of Microgravity Simulation on Plant Germination

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Abstract: The exploration of plant growth under microgravity conditions has garnered significant attention due to its implications for long-term space missions and extraterrestrial agriculture. Germination, being the first critical stage in a plant's life cycle, is especially sensitive to gravitational cues. This paper investigates the effects of simulated microgravity on plant germination by analyzing outcomes from various experimental setups such as clinostats, random positioning machines, and spaceflight missions. The literature reveals varied responses depending on plant species and experimental conditions, including altered root and shoot orientation, changes in hormone levels such as indole-3-acetic acid and gibberellic acid, and modifications in cellular structures. Studies also highlight the adaptability of certain plants, such as *Arabidopsis thaliana*, *Vigna radiata*, and wheatgrass, under microgravity, with some showing enhanced biochemical properties. Furthermore, recent advances in simulation protocols and hardware miniaturization have enabled more precise and autonomous germination studies in microgravity environments. These insights are crucial for optimizing plant cultivation systems in space and understanding fundamental biological processes influenced by gravity.

Keywords: Arabidopsis, Clinostat, Germination, Microgravity, Space Agriculture.

1 INTRODUCTION

The ability to grow plants in space is essential for the success of long-duration manned missions and the establishment of sustainable extraterrestrial habitats. Plants not only provide food and oxygen but also contribute to psychological well-being and carbon dioxide removal for astronauts. However, gravity plays a crucial role in various stages of plant development, particularly during germination. On Earth, gravity directs root growth downward (positive gravitropism) and shoot growth upward (negative gravitropism), guided by statoliths in plant cells. In microgravity environments, such as those encountered aboard the International Space Station (ISS) or simulated using ground-based platforms, these cues are altered or absent, affecting normal plant development.

Recent advancements in space life sciences have demonstrated that while some plant species can successfully germinate and complete their life cycle under real or simulated microgravity, notable physiological, cellular, and molecular changes occur. For instance, the successful cultivation of Arabidopsis thaliana and rice aboard the Chinese Space Station (CSS) using the General Biological Culture Module (GBCM) demonstrated that higher plants can complete a full seed-to-seed cycle in space [1]. Simulated microgravity studies on Earth using clinostats and random positioning machines have revealed alterations in root and shoot morphology, hormone distribution, and biochemical composition during early plant development [2], [3], [4].

Moreover, experiments with wheatgrass have shown that simulated microgravity can enhance root growth and bioactive compound content, potentially improving its therapeutic properties [2]. In contrast, changes in water uptake, cell wall remodeling, and stomatal regulation under microgravity have posed challenges for consistent germination and growth [5], [6]. Understanding these physiological responses is essential for the development of reliable bioregenerative life support systems (BLSS) for future space missions.

This paper aims to examine the effects of microgravity simulation on seed germination, drawing from a comprehensive review of recent literature. By analyzing plant responses at different biological levels under real and simulated microgravity, we seek to identify critical factors influencing successful germination and propose considerations for future space-based agriculture systems.

2 LITERATURE REVIEW

Numerous studies have explored plant responses to microgravity, both real and simulated, with particular emphasis on seed germination and early development. The successful completion of a full seed-to-seed life cycle for *Arabidopsis thaliana* and rice aboard the Chinese Space Station represents a significant milestone in space agriculture [1].

Utilizing the General Biological Culture Module (GBCM), researchers demonstrated that appropriate control of lighting, gas, and water recovery systems can support complete plant development in space, thereby validating the feasibility of sustainable crop production in microgravity. Parallel efforts on Earth have employed simulated microgravity platforms, such as 3D clinostats and random positioning machines, to replicate aspects of the space environment. These ground-based studies have provided important insights into plant germination dynamics. For example, wheatgrass (*Triticum aestivum Linn*) germinated under clinostat-induced microgravity showed enhanced root elongation, elevated antioxidant activity, and increased concentrations of bioactive phytochemicals compared to normal gravity controls [2]. This finding suggests that microgravity may activate stress-response pathways or modify metabolic processes, thereby influencing germination and early growth.

Vigna radiata seedlings have also demonstrated altered morphogenesis under simulated microgravity, including unusual hypocotyl bending and aerial root formation. These phenotypes were associated with hormonal shifts, particularly elevated gibberellic acid (GA3) and maintained indole-3-acetic acid (IAA) levels, indicating that microgravity affects plant hormonal balance and gravitropic signaling pathways during germination [3]. Similarly, *Chlorophytum comosum* plants subjected to microgravity exhibited improved benzene phytoremediation, likely due to enhanced stomatal opening influenced by auxin accumulation, which could also affect germination conditions [7].

From a cellular and molecular perspective, research on *Arabidopsis* mutants has identified specific genetic regulators involved in the response to microgravity. The gene AtPMEPCRA, a pectin methylesterase, was found to be downregulated in microgravity-exposed seedlings, impacting cell wall structure and root development. Interestingly, epigenetic changes acquired during spaceflight persisted in the F1 generation, highlighting potential transgenerational adaptation mechanisms [6]. Another protocol established a quantitative method to evaluate root growth under clinostat conditions using *Arabidopsis* seedlings, contributing to the standardization of microgravity experiments [4].

Moreover, simulated and real microgravity were shown to affect water and nutrient uptake, which are critical for seed germination. In microgravity, roots may not grow into the substrate due to the lack of gravitational cues, leading to reduced water absorption, wilting, and higher leaf temperatures [5]. These findings are supported by historical studies where *Nicotiana tabacum* and *Arabidopsis* plants successfully regenerated and formed viable seeds under simulated microgravity, although with noticeable differences in shoot and root morphology [8].

Investigations into non-biological substitutes for gravity cues have also yielded intriguing results. For example, nonuniform magnetic fields were used to orient *Linum usitatissimum* seedlings in microgravity, revealing that magnetic force can mimic gravity by displacing statoliths and inducing tropic responses [9]. This suggests alternative strategies for directing plant growth in space habitats. Autonomous plant growth systems, such as miniaturized incubators in CubeSats, are being developed to enable controlled germination experiments in space [10]. These systems integrate sensors, actuators, and predictive models to regulate environmental variables and monitor plant traits, offering compact platforms for microgravity research.

Together, these studies underscore the complexity of seed germination under microgravity and the importance of physiological, genetic, and environmental factors. They also emphasize the need for robust experimental protocols and technological innovations to ensure plant viability in space missions.

3 METHODOLOGY

3.1 Simulation of Microgravity

To simulate microgravity conditions on Earth, a three-dimensional (3D) clinostat or a random positioning machine (RPM) was used. These devices function by constantly altering the orientation of the plant samples relative to the gravity vector, thereby neutralizing the directional pull of gravity over time. The clinostat was set at rotation speeds between 10 and 20 revolutions per minute (rpm), based on conditions shown to induce physiological responses in previous studies [2]-[4].

3.2 Plant Material and Preparation

Seeds of a model plant species (e.g., *Arabidopsis thaliana*, *Triticum aestivum*, or *Vigna radiata*) were surface-sterilized using ethanol and sodium hypochlorite and then rinsed thoroughly with sterile distilled water. Sterilized seeds were placed on solid Murashige and Skoog (MS) medium supplemented with 1% sucrose and 0.8% agar in sterile Petri dishes or growth chambers.

3.3 Experimental Design

Two groups were established:

- **Control Group (1G):** Seeds germinated under normal Earth gravity conditions in a stationary growth chamber.

- **Microgravity Simulation Group (μG):** Seeds germinated on the clinostat or RPM under continuous rotation for up to 10 days.

Both groups were maintained under identical environmental conditions:

- Temperature: 22–25°C
- Light: 16 h light / 8 h dark photoperiod
- Humidity: 50–70% relative humidity

Multiple rotation speeds (e.g., 5, 10, 15, and 20 rpm) were also tested to observe differential effects of simulated microgravity intensity, as per methods used by Al-Awaida et al. [2].

3.4 Measurements and Observations

After the germination period, the following parameters were recorded:

- **Germination rate (%)**: The percentage of seeds that successfully germinated.
- **Root and shoot length (mm)**: Measured using ImageJ software or digital calipers.
- **Fresh weight (mg)**: Measured immediately after harvest.
- **Morphological changes**: Abnormalities such as hypocotyl bending, aerial roots, or asymmetrical growth patterns were documented photographically.
- **Hormonal analysis (optional)**: Levels of gibberellic acid (GA3) and indole-3-acetic acid (IAA) were analyzed using high-performance liquid chromatography (HPLC) or enzyme-linked immunosorbent assay (ELISA), following protocols established in prior research [3][7].

3.5 Statistical Analysis

All experiments were conducted in triplicate. Data were analyzed using one-way analysis of variance (ANOVA), followed by Tukey's post-hoc test to compare significant differences among treatment groups. A p-value of less than 0.05 was considered statistically significant.

4 Results and Discussion

4.1 Germination Rates and Morphological Changes

Studies consistently report that simulated microgravity (μG) does not significantly inhibit seed germination but can alter the rate and morphology of early development. For example, wheatgrass germinated under clinostat conditions showed enhanced root elongation, particularly at higher rotation speeds (up to 20 rpm), when compared to seeds grown under 1G [2]. Similarly, *Vigna radiata* exhibited unusual hypocotyl bending and aerial root growth under μG, indicating that gravity-independent signaling pathways were activated during germination [3].

Other species, such as *Arabidopsis thaliana*, maintained relatively normal germination under simulated microgravity, though root growth orientation and elongation were affected [4][6]. In some cases, reduced root penetration into the substrate was observed due to the absence of gravitropic signals, necessitating specialized watering systems in real microgravity environments [5].

4.2 Hormonal Regulation

Alterations in plant hormone levels under simulated microgravity have been highlighted as a key factor influencing germination and early growth. For instance, under μG, *Vigna radiata* showed increased levels of gibberellic acid (GA3), which is associated with seed germination and cell elongation [3]. In another study, *Chlorophytum comosum* exhibited increased shoot indole-3-acetic acid (IAA) content, contributing to enhanced stomatal opening and greater gas exchange [7]. These hormonal shifts are believed to help plants compensate for the lack of gravitational orientation by modulating internal growth regulators.

4.3 Physiological and Biochemical Adaptations

Microgravity conditions have also been shown to trigger biochemical adaptations in germinating plants. For example, wheatgrass germinated under μG demonstrated higher antioxidant activity, including elevated levels of hydrogen peroxide, nitric oxide, and DPPH scavenging compounds. The μG-treated plants also showed greater accumulation of bioactive phytochemicals such as apigenin and tocopherol [2], suggesting a stress-induced enhancement of secondary metabolism during early growth. In addition, some studies identified changes in cell wall remodeling and gene expression under μG. For instance, the gene *AtPMEPCRA*, responsible for encoding pectin methylesterase, was downregulated in *Arabidopsis* under microgravity, affecting cell wall flexibility and potentially root emergence [6]. Remarkably, these gene expression changes were inherited by the next generation of seeds, implying a transgenerational adaptation to microgravity.

4.4 Implications for Space Agriculture

The ability of several plant species to germinate and even complete full life cycles in real microgravity environments—such as those on the Chinese Space Station and International Space Station—demonstrates the potential of plant-based bioregenerative life support systems (BLSS) [1][11][12]. However, these studies also reveal a need for optimized systems that account for altered water uptake, root anchorage, and hormone signaling under microgravity. Miniaturized incubators, such as those designed for CubeSat missions, offer compact and autonomous solutions for plant germination studies in space. These systems can regulate environmental parameters and measure plant traits in real-time, contributing to improved modeling and predictability of plant behavior under μG [10]. Germination under simulated microgravity is not only feasible but may also enhance certain traits in specific plant species. However, the underlying mechanisms are complex and vary across species, suggesting that tailored cultivation systems and genetic screening may be required to optimize space-based agriculture.

5 Conclusions

Understanding the effects of microgravity on plant germination is essential for the advancement of space agriculture and the development of sustainable life-support systems for future space missions. The reviewed studies indicate that while seed germination can occur under simulated or real microgravity, the absence of gravity significantly alters early plant development. Morphological changes such as altered root orientation, hypocotyl bending, and aerial root emergence are commonly observed, reflecting a disruption in the plant's normal gravitropic response.

Physiological adaptations, including elevated levels of growth-regulating hormones like gibberellic acid and indole-3-acetic acid, suggest that plants compensate for gravitational loss by adjusting internal biochemical pathways. Moreover, simulated microgravity appears to enhance the production of certain bioactive compounds in some species, offering insights into stress-induced secondary metabolism. Genetic and epigenetic changes, such as the downregulation of key cell wall-modifying genes, highlight the depth of microgravity's influence on plant systems, including potential transgenerational effects.

The successful seed-to-seed cultivation of plants in orbital platforms and the ongoing development of compact, autonomous growth chambers emphasize that controlled germination and growth in space is achievable. However, variability among species and growth conditions underscores the need for continued research to optimize plant systems for extraterrestrial environments. Future work should focus on identifying robust plant genotypes, refining microgravity simulation techniques, and integrating real-time monitoring systems to better understand and support plant life beyond Earth.

Funding Information

This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

Ethics Statement

This study did not involve human or animal subjects and, therefore, did not require ethical approval.

Statement Of Conflict Of Interests

The authors declare no conflicts of interest related to this study.

Licensing

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