

Cultivation of Microgreens in a Nutrient-Substrate-Soil-less Microsystem Under Simulated Lunar Gravity: Growth Patterns, Morphological Traits, and Physiological Responses

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Abstract

The successful cultivation of fresh vegetables in space as a dietary supplement is critical for supporting long-duration missions, where the degradation of nutrients in pre-packaged foods could damage astronaut health. This study explores the growth patterns, morphological traits, and physiological responses of 5 (five) microgreen varieties - Broccoli (*Brassica rapa var cymosa*), Radish (*Raphanus sativus var sativus*), Mustard (*Sinapis alba*), Alfalfa (*Medicago sativa*), and Mungbean (*Vigna radiata*) - under simulated lunar gravity (lun-g) conditions using a Random Positioning Machine (RPM) and a minimalistic Nutrient-Substrate-Soil-less Microsystem (NSLM), comparing these development performance features with terrestrial controls. The results demonstrated that all selected microgreen varieties successfully developed in the system proposed. The samples exposed to lun-g exhibited accelerated growth compared to the terrestrial controls, achieving full maturity in only four days. Additionally, increased fresh mass of 107.32% for Broccoli (*Brassica rapa var cymosa*) and 9.30% for Mungbean (*Vigna radiata*) as maximum and minimum values were observed. A significant increase in stem length was observed in the NSLM samples under lun-g, namely, 309.65% Mustard (*Sinapis alba*) and 62.13% Mungbean (*Vigna radiata*) - maximum and minimum values - compared to their respective terrestrial controls. No other significant morphological alterations were observed, indicating that the five (5) selected varieties of microgreens can effectively adapt to low-resource environments and lun-g gravity conditions. The findings of this study demonstrate the potential of minimal-growing systems to cultivate fresh leafy vegetables in space, eliminating the need for complex substrates and nutrient management, reducing the cultivation area and optimizing the need for irrigation water, as well as minimizing the related logistical challenges.

1. INTRODUCTION

Fresh vegetables' cultivation aboard spacecraft requires innovative yet practical solutions to enable the *in-situ* production of high-nutritional food, particularly for long-duration space missions. Developing a sustainable Controlled Ecological Life Support System (CELSS) is one of today's key space challenges, not only for meeting astronauts' dietary requirements, but also for aiding the physiological and psychological issues associated respectively with exposure to space radiation and microgravity, and extended periods in isolated and confined environments.

To date, pre-packaged foods are the primary nutrition source for crew members during space missions. Although diverse and safe, Cooper et al. (2017) demonstrated that essential nutrients degrade over time under usual space missions' storage conditions. Their study found that vitamins B1 and C degrade to inadequate concentrations within three (3) years when stored at 21°C. Although vitamins A, B9, and B12 also exhibit a decline in concentration, the reduction is less pronounced. Previous studies have also found similar degradation effects on packaged space food stored at 21°C [Zwart et al. (2009); Catauro et al. (2012)]. More recently, Pittia et al. (2023) reiterated that prolonged storage can significantly reduce the bioavailability and bioaccessibility of nutrients in packaged space food due to degradation reactions, compromising both the nutritional and sensory quality thereof. In this field of study, it became clear that

cultivating fresh, nutrient-rich leafy vegetables on spacecraft can serve as an important dietary supplement for astronauts, addressing the nutritional and sensorial gaps left by the degradation of packaged space food. The benefits of edible leaves' cultivation on spacecraft can go beyond nutrition: Odeh et al. (2017) demonstrated that human interaction with plants, or biophilia, can mitigate the possible psychosocial and neurocognitive detrimental consequences associated with long-duration space missions, offering a countermeasure against stress, isolation, and cognitive fatigue. In 2021, Neilson et al. (2021) published a mini-review of the growing evidence body that supports the biophilia hypothesis in space environments. This mini-review reiterates how exposure to natural elements, even simulated ones, can mitigate the detrimental psychological impacts caused by isolation and long-term confinement. This report concludes that integrating biophilic design, such as plants, natural colors, and digital images of nature, can significantly help reduce stress and mitigate cognitive fatigue during prolonged space missions.

Developing an optimal sustainable food cultivation system for space, namely, a *Controlled Ecological Life Support System* (CELSS) or *Bioregenerative Life Support System* (BLSS), presents significant challenges. Key cultivation factors, such as (but not limited to) vapor pressure deficit (VPD), light intensity and quality, temperature regulation, water availability, and substrate selection, are critical to modulating plant performance. These factors might have unexpected effects on plant development when acting under space environmental conditions.

Understanding the impact of direct space factors and limitations, such as (but not limited to) microgravity, radiation, minimum cultivation area or volume, humidity, ventilation, atmospheric composition, and quantity and quality of light, is essential for optimizing water and nutrient uptake in plants in extended space missions. In addition, research is needed to evaluate and improve the effectiveness of current space cultivation systems in terms of the dynamics of growth substrates and water delivery. All these factors can directly influence plant morphogenesis, physiological processes, productivity, yield, and the nutritional value and safety of the food produced.

A recent publication by De Micco et al. (2023) summarizes insightfully the open scientific questions and knowledge gaps that must be investigated to improve crop cultivation and food production in space. Central to these challenges is the need to test and identify the most effective and sustainable solutions when it comes to substrates, efficient water and nutrient delivery, atmosphere management, and lighting.

In selecting species or cultivar varieties for space cultivation, priority should be given to those with high nutritional value and health-promoting properties, minimum cultivation requirements, and high resistance to space-related stressors. Considerations should also include species that require minimal crew work and effective protocols designed to ensure food safety while reducing processing needs. Taking these requirements into account, microgreens have emerged as a species with great potential for space crops in recent decades.

Microgreens can be described as seeds' germination that have fully grown genuine leaves and non-senescent cotyledons but are harvested or collected before the development of roots [Lone et al. (2024);

Antony, V. (2020)]. Due to their fast-growing features with short production cycles, high yield index, small plant size, high nutrient content, minimal resources, growing area, and minimal photon flux requirements [Izzo et al. (2023); Teng et al. (2022); De Micco et al. (2023)], space companies and academic research institutes have increased interest in the study of microgreens as food supplements for astronauts [De Francesco et al. (2023); Dawra et al. (2023)].

In addition to their nutritional values, all the varieties of microgreens available and grown on Earth potentially have health-promoting phytochemicals in greater or lesser concentrations. Several works have investigated microgreens' health-promoting phytochemicals, how they relate to different microgreens' varieties, and the modulation of environmental or cultivation factors, reporting that these leafy greens contain higher concentrations of nutrients compared to their mature counterparts. There are some good books and works on this subject, such as *Microgreens: A Guide to Growing Nutrient-Packed Greens* by Franks, E., Richardson, J. (2009); *Microgreen nutrition, food safety, and shelf life: A review* by Turner, E. R. et al. (2020); *Microgreens for Nutritional Security* by Kumari, Veenita & Junuthula, Shirisha & Mandapaka, Ravi. (2023), and *Microgreens and novel non-thermal seed germination techniques for sustainable food systems: a review* by Bhabani, M. G. et al. (2024).

With a view to aeronautical applications, the available studies on microgreen cultivation in space and space-simulated environments reveal interesting findings and areas for improvement (presented below). These works will be discussed in the light of the findings of the present study, and by scrutinizing developments and technological gaps that still exist.

The present study aims to investigate microgreens' potential cultivation and development in response to simulated lunar gravity (lun-g) and the designed Nutrient-Substrate-Soil-less Microsystem (NSLM), using only Milli-Q water, without any plant substrate, no nutrient input, and with no sunlight or special lighting setup.

In selecting the microgreens' varieties for this study, we focused on the varieties that could provide the vitamins and nutrients that help astronauts cope with health challenges in space - namely, but not limited to - vitamin B (mainly B1), C, A, E, and K; minerals Calcium, Magnesium, Iron, Potassium, Zinc, and polyphenols (primarily antioxidants). We therefore elected two microgreens' Families - *Brassicaceae* and *Fabaceae* - for the present investigation. To select the varieties of microgreens tested, the aforementioned study by Izzo et al. (2023) was considered. Their study developed an algorithm to compare microgreens' genotypes for productivity and nutritional content, which is good for space applications. The approach led to a prioritized ranking, highlighting *Brassicaceae* varieties as the top candidates based on their terrestrial yield and phytonutrient profile. The study from Goyal, Arvind & Brahma, Birendra. (2014) supports that *Fabaceae* species are rich in proteins and Vitamin B1.

The present study investigated the growing patterns of five (5) microgreens' varieties - Broccoli (*Brassica rapa var cymosa*), Radish (*Raphanus sativus var sativus*), Mustard (*Sinapis alba*), Alfalfa (*Medicago sativa*), and Mungbean (*Vigna radiata*) - in response to the NSLM conditions and simulated lunar gravity (lun-g). The proposed research experiment was carried out in three (3) consecutive cultivation cycles.

Each of these cycles comprised five (5) NSLM samples exposed to simulated lunar gravity (lun-g) and the same number of control NSLM samples, grown under terrestrial gravity. During the cultivation cycles, the main growth parameters were monitored daily, such as growth pattern, morphological characteristics, and physiological responses, including fresh weight, stem length, and width, thus comparing the responses of the NSLM samples under simulated lunar gravity (lun-g) and control conditions.

By understanding the feasibility of the proposed Nutrient-Substrate-Soil-less Microsystem (NSLM) and respective microgreens' development, we aim to gain insights into simplified space-cultivation systems. These could minimize the need for cultivation area, complex substrates, nutrients, and lighting setups in space, which are crucial considerations for long-duration space missions.

2. MATERIALS AND METHODS

2.1 Plant Material

Five (5) microgreen varieties were selected: Broccoli (*Brassica rapa var cymosa*), Radish (*Raphanus sativus var sativus*), Mustard (*Sinapis alba*), Alfalfa (*Medicago sativa*), and Mungbean (*Vigna radiata*). Seeds were purchased from a local provider and stored in their original packaging in a dark environment at room temperature until use.

2.2 Experimental Setup

The proposed research experiment was carried out in three (3) consecutive cultivation cycles, as described below. Each of these cycles comprised ten (10) samples of one gram (1 g) of microgreen seeds, carefully weighed and placed into individual glass containers. After placing the seeds into the glass container, they were sealed with Parafilm®. Small holes were made in the sealing Parafilm®, using a sterile lab needle. Please note that the holes were made from outside to inside the Parafilm®, aiming to ensure minimal airflow while also preventing the irrigating water from coming from inside the glass container (Fig. 1).

As mentioned before, one gram (1 g) of microgreen seeds was carefully weighed and placed in individual sealed glass containers. Two milliliters (2 mL) of Milli-Q water were then added to each container using a micropipette, thus establishing the proposed NSLMs.

On the first day of the research experiment, the ten (10) samples were prepared so that five (5) samples were exposed to simulated lunar gravity (lun-g) and five (5) samples were the terrestrial controls. The NSLMs were then grouped by microgreen species and arranged on a plastic support to securely hold them on the desktop of the Random Positioning Machine RPM 2.0 (Yuri) during operation (Fig. 02).

To maintain a constant humidity level, the RPM was briefly interrupted every 24 (twenty-four) hours to manually add a further two milliliters (2 mL) of Milli-Q water to each NSLM, using a micropipette to ensure accuracy.

The three (3) cultivation cycles were identical in terms of environmental conditions. The ten (10) NSLM samples (lun-g and terrestrial controls) were kept in a closed environment with a temperature of 22 ± 2°C, relative humidity of 60–70% (World Weather Online, Climate Data), and a photoperiod of sixteen (16) hours of light and eight (8) hours of darkness (See Table 01 below).

It is worth emphasizing that Milli-Q water was tested in this research experiment because of the advantages its high purity offers. Milli-Q water is free of contaminants such as particles, bacteria, endotoxins, and volatile organic compounds. This level of purity ensures that no foreign substances from the irrigation interfere with the experimental results.

2.3 Data Collection

The RPM was briefly halted every 24 (twenty-four) hours to enable NSLM sample photography and data collection. The main growth parameters were monitored, such as growth pattern, morphological characteristics, and physiological responses, including fresh weight, stem length, and width, thus comparing the responses of the NSLM samples under simulated lunar gravity (lun-g) and control conditions.

To monitor the aforementioned growth parameters, each NSLM sample (lun-g and terrestrial control) was carefully drained using a standard clean paper filter. The drained material was then weighed to determine its fresh weight (FW), expressed in grams (g). At every data collection, a plant unit was carefully removed from each of the NSLM samples for precise measurements of Stem Length (SL) and Stem Width (SW), recorded with a caliper.

The microgreen varieties and parameters analyzed in this study are summarized in Table 01 below.

Table 01
Microgreen varieties, experiment conditions, and parameters analyzed in this study.

MICROGREEN VARIETIES	EXPERIMENT CONDITIONS	DRY SEED WEIGHT (g)	MILLI-Q WATER ADDED (mL)	PARAMETERS ANALYZED
<i>Brassica rapa</i> var <i>cymosa</i> , <i>Raphanus sativus</i> var <i>sativus</i> , <i>Sinapis alba</i> , <i>Medicago sativa</i> , <i>Vigna radiata</i>	Air temperature (T) 22 ± 2°C, 16 hours light and 8 hours dark, relative humidity of 60–70%, Simulated Lunar Gravity (lun-g), Control	1	10	Fresh weight (FW, g), Stem length (SL, mm), Stem width (SW, mm)

3. RESULTS & DISCUSSION

3.1 Experimental Setup: The NSLM.

Figure 03 below displays the photographs of the NSLM samples taken every 24 (twenty-four) hours consistently, to compare the morphological traits of the microgreen varieties under simulated lunar

gravity (lun-g) and respective terrestrial control.

The first result of the present study states that all microgreen varieties, both those subjected to simulated lunar gravity (lun-g) and their respective terrestrial controls, were able to develop in the proposed NSLM conditions. Furthermore, it was observed that the NSLM samples not only grew, but when subjected to simulated lunar gravity (lun-g), they exhibited accelerated development and maturity compared to the terrestrial controls. The NSLM samples under simulated lunar gravity (lun-g) reached full maturity in only four (4) days.

Figure 03 shows that all the NSLM samples (lun-g and control) of each microgreen variety tested were able to effectively germinate, or at least start germinating, within the four (4) days of the experiment. Plant seeds contain nutrient reserves sufficient for the initial stages of germination and early growth. This nutrient reserve, primarily composed of carbohydrates, proteins, and lipids, supports the seedling until plants can photosynthesize and obtain nutrients from external sources [Weitbrecht et al. (2011)]. Microgreens, like any other plant, can complete germination and reach the first true-leaf stage without external nutrient supplementation, as demonstrated by multiple studies [Moraru et al. (2021); Lone, J. K., & Pandey, R. (2024); Ortiz et al. (2024)]. Milli-Q water, while ultrapure, contains no essential nutrients or minerals for proper plant growth. Additionally, Milli-Q water lacks buffering capacity, which may lead to rapid pH changes and osmotic stress for plants [Blumwald et al. (2000)]. Despite this, the photographs in Figure 02 show that all the microgreen varieties tested can grow using Milli-Q water alone. No damaging stress was observed, neither in the simulated lunar gravity (lun-g) nor in the terrestrial controls.

For space applications, given this first result, the proposed Nutrient-Substrate-Soil-less Microsystem (NSLM) could offer advantages over more complex plant compartments without prejudice to germination or growth of the microgreens. One of the advantages to point out is that using Milli-Q water alone could reduce the need for storage and handling of nutrient solutions. Moreover, Milli-Q water ultrapurity can minimize the risk of introducing contaminants or pathogens that could compromise the microgreen growth environment or crew health. Additionally, eliminating the need for soil or substrates saves space, reduces the mass of the plant compartment, and avoids the complications of managing soil-borne pathogens or contamination in a closed environment. To date, plant experiments in space have mainly been done with a granular media or gel- and mat-like substrate to hold the seeds/plants in place and controlled-release fertilizer pellets with the addition of water [De Micco et al. (2023)].

With the reduced complexity of the plant compartment proposed and its simple design without the need for nutrient delivery systems, complex lighting setups, or substrate management, the NSLM is less prone to mechanical failures and easier to maintain, reducing the crew time required. This minimalistic setup also requires less physical space, and, therefore, with no substrates, nutrients, or complex lighting systems, the overall mass of the plant compartment system is significantly reduced, lowering launch costs.

3.2 The Impact of Simulated Lunar Gravity (lun-g) on Growth Patterns, Morphological Traits, and Physiological Responses.

3.2.1 Growth and Maturity

The photographs shown in Figure 03 reveal that all five (5) NSLM microgreen varieties subjected to simulated lunar gravity (lun-g) presented accelerated growth compared to their respective terrestrial controls. This enhancement in growth is consistent with other studies that indicate that reduced gravity can have a stimulatory effect on plant growth, which could lead to enhancing germination and development. In 2021, a study by Oluwafemi et al. (2021) tested Okra (*Abelmoschus esculentus*) seeds on agar-agar as substrate, using a horizontal clinostat to simulate microgravity (80 rpm). This study demonstrated a growth rate increase of okra under simulated microgravity by 14.01%. In 2024, Jagtap et al. (2024) reported an enhanced growth of monocotyledonous rice (*Oryza sativa* 'PRH-10') and dicotyledonous mungbean (*Vigna radiata* (L.) Wilczek) on agar gel, using a clinostat with one rotation axis (2 rpm).

Visual observations depicted in Figure 03 indicated that the growth was significantly higher in the *Raphanus sativus* var *sativus* and *Medicago sativa* under simulated lunar gravity (lun-g) when compared to their respective terrestrial control. The smallest differences in growth were observed in the *Sinapis alba* and *Vigna radiata*, suggesting that there is an interaction between varieties and the environmental conditions to which they are exposed.

The present study also reports that, beyond the enhanced growth, all the NSLM varieties under simulated lunar gravity (lun-g) presented faster plant maturity, achieving full maturity in only four (4) days. Accelerated growth and maturity observed in NLSM samples under simulated lunar gravity (lun-g) conditions raise questions about how the proposed cultivation conditions affect the utilization of plant nutrient reserves. Future studies should investigate whether enhanced microgreen growth under simulated lunar gravity (lun-g) conditions is related to accelerated nutrient consumption from the reserves, leading to earlier maturity (and depletion).

3.2.2 Fresh weight (FW)

Table 2 and Figure 04 below present the Fresh Weigh (FW) response for each microgreen variety studied over the four (4) days of the research experiment, in the samples NLSM under simulated lunar gravity (lun-g) and terrestrial control.

Table 02: Fresh Weigh (FW) response for Broccoli (*Brassica rapa* var *cymosa*), Radish (*Raphanus sativus* var *sativus*), Mustard (*Sinapis alba*), Alfalfa (*Medicago sativa*), and Mungbean (*Vigna radiata*), for samples NLSM under simulated lunar gravity (lun-g) and terrestrial control.

MICROGREEN VARIETY	DAY 1 FW (g)	DAY 2 FW (g)	DAY 3 FW (g)	DAY 4 FW (g)	Total FW Diff. (%)
Broccoli (lun-g)	2.5008±1.05	3.0496±0.83	5.0717±0.99	6.3043±0.42	107.32
Broccoli Control	2.4197±1.11	2.7299±1.26	2.8763±1.16	3.0409±1.06	
Radish (lun-g)	2.1061±0.56	3.8099±0.27	5.2206±0.52	6.3479±0.49	62.34
Radish Control	2.0260±0.13	2.5049±0.53	3.3599±0.72	3.9103±1.00	
Mustard (lun-g)	2.9368±0.35	3.9889±0.29	5.3441±0.48	6.6475±0.72	60.67
Mustard Control	2.5153±0.39	3.5624±0.48	3.8053±0.89	4.1372±1.31	
Alfalfa (lun-g)	3.0156±0.61	4.4338±0.87	5.5333±0.57	7.1486±0.40	39.87
Alfalfa Control	3.3110±0.34	3.5514±0.55	3.7466±0.46	5.1107±2.32	
Mungbean (lun-g)	2.6417±0.073	4.4338±0.088	4.9932±0.16	5.8050±0.51	9.30
Mungbean Control	2.7904±0.39	3.5514±0.55	4.8621±0.39	5.3113±0.27	

Table 2 and Figure 04 revealed that all five (5) microgreen varieties NSLM under simulated lunar gravity (lun-g) exhibited higher Fresh Weight (FW) compared to their respective terrestrial controls. The highest to lowest difference in total Fresh Weight (FW) was found in the following order: 107.32% Broccoli (*Brassica rapa var cymosa*), 62.34% Radish (*Raphanus sativus var sativus*), 60.67% Mustard (*Sinapis alba*), 39.87% Alfalfa (*Medicago sativa*), and 9.30% Mungbean (*Vigna radiata*).

These total Fresh Weight (FW) responses agree with the results obtained by De Francesco et al. (2025), which reported that *Brassica rapa L.* microgreens subjected to simulated microgravity conditions (μg) demonstrated significantly higher fresh weight than those grown under standard Earth gravity (1 g). Their findings suggest that reduced gravity may favor early biomass accumulation in microgreens. Liu et al. (2018) also reported that plants grown in s0-g produced higher biomass than at 1-g. They observed that a biomass increase of up to 40% was reported in roots and hypocotyls of *Veronica arvensis* grown on a clinostat.

Further studies are needed to understand the physiological and molecular mechanisms driving higher Fresh Weight (FW) on microgreens under simulated lunar gravity (lun-g).

3.2.3 Stem Length

Table 03 and Figure 05 below present the Stem Length (SL) response of each microgreen variety studied over the four (4) days of the research experiment, in the samples NLSM under simulated lunar gravity (lun-g) and terrestrial control.

Table 03: Stem Length (SL) response for Broccoli (*Brassica rapa var cymosa*), Radish (*Raphanus sativus var sativus*), Mustard (*Sinapis alba*), Alfalfa (*Medicago sativa*), and Mungbean (*Vigna radiata*), for samples NLSM under simulated lunar gravity (lun-g) and terrestrial control.

MICROGREEN VARIETY	DAY 1 SL (mm)	DAY 2 SL (mm)	DAY 3 SL (mm)	DAY 4 SL (mm)	Total SL Diff. (%)
Broccoli (lun-g)	2.85±0.43	9.62±1.23	19.88±6.55	28.17±8.22	273.11
Broccoli Control	1.80±0.35	4.34±0.25	5.74±1.67	7.55±3.07	
Radish (lun-g)	6.41±0.96	20.06±2.85	36.65±8.73	44.94±12.38	111.46
Radish Control	4.24±0.17	8.23±3.73	12.10±7.06	21.25±7.02	
Mustard (lun-g)	6.18±1.02	17.65±5.80	21.41±2.92	27.58±3.12	309.65
Mustard Control	3.84±1.44	5.94±1.40	5.56±0.82	6.73±0.52	
Alfalfa (lun-g)	5.05±1.71	10.52±4.54	16.41±1.64	16.77±2.54	106.83
Alfalfa Control	3.89±0.67	4.60±1.27	6.59±1.38	8.11±1.63	
Mungbean (lun-g)	9.75±1.89	18.54±4.20	28.76±1.89	37.56±2.47	62.13
Mungbean Control	9.54±1.93	11.45±3.55	21.34±2.08	23.17±5.33	

Table 3 and Figure 05 revealed that all five (5) microgreen varieties NSLM under simulated lunar gravity (lun-g) exhibited a longer Stem Length (SL) compared to their respective terrestrial controls. The highest to lowest difference in total Stem Length (SL) was found in the following order: 309.65% Mustard (*Sinapis alba*), 273.11% Broccoli (*Brassica rapa var cymosa*), 111.46% Radish (*Raphanus sativus var sativus*), 106.83% Alfalfa (*Medicago sativa*), and 62.13% Mungbean (*Vigna radiata*).

The responses of total Stem Length (SL) agree with the results obtained by Shen et al. (2018). The study presented nine strains of lettuce growth in a space vegetable cultivation facility onboard the Tiangong 2 Spacelab under spaceflight microgravity. Their study reported taller plants generated in the condition of microgravity, 'possibly due to two reasons. Firstly, rooting substances needed by the plant, in particular growth hormone, fastened in the top part without gravity, which accelerated the increase of the plant's height condition. Secondly, in spaceflight μ -g condition, water and nutrition evenly disperse into the rooting substrate, however, gravity drives water to the bottom of the rooting substrate on Earth. Therefore, the water and nutrition were more abundant around roots in space than on Earth, which seems to be more beneficial to plant growth and development.' In this direction, Hoson et al. (2013) results obtained in spaceflight microgravity demonstrated a 16% increase in whole hypocotyl length under microgravity conditions for Arabidopsis samples, therefore confirming stimulation of elongation growth under microgravity conditions in space at the cellular level. Matía et al. (2010) study also

described significantly longer shoot lengths for *Arabidopsis thaliana* (*Columbia-0* and *Wassilewskija*) seedlings grown in spaceflight and simulated microgravity.

3.2.4 Stem Width

Table 4 and Figure 06 below present the Stem Width (SW) response of each microgreen variety studied over the four (4) days of the research experiment, in the samples NLSM under simulated lunar gravity (lun-g) and terrestrial control.

Table 04: Stem Width (SW) response for Broccoli (*Brassica rapa var cymosa*), Radish (*Raphanus sativus var sativus*), Mustard (*Sinapis alba*), Alfalfa (*Medicago sativa*), and Mungbean (*Vigna radiata*), for samples NLSM under simulated lunar gravity (lun-g) and terrestrial control.

MICROGREEN VARIETY	DAY 01 SW (mm)	DAY 02 SW (mm)	DAY 03 SW (mm)	DAY 04 SW (mm)	Total SW Diff. (%)
Broccoli (lun-g)	1.25±0.21	1.75±0.10	2.26±0.18	2.69±0.06	33.61
Broccoli Control	1.12±0.14	1.38±0.23	1.92±0.09	2.01±0.22	
Radish (lun-g)	0.97±0.06	1.26±0.20	1.31±0.07	1.50±0.06	31.96
Radish Control	0.74±0.21	1.01±0.09	1.14±0.17	1.14±0.13	
Mustard (lun-g)	0.38±0.37	0.47±0.43	0.48±0.61	0.67±0.67	41.35
Mustard Control	0.33±0.35	0.34±0.34	0.45±0.54	0.48±0.56	
Alfalfa (lun-g)	0.85±0.10	0.89±0.09	1.41±0.06	1.41±0.07	29.12
Alfalfa Control	0.20±0.35	0.28±0.30	0.99±0.12	1.09±0.03	
Mungbean (lun-g)	1.25±0.21	1.75±0.10	2.26±0.18	2.69±0.06	33.61
Mungbean Control	1.12±0.14	1.38±0.23	1.92±0.09	2.01±0.22	

The differences in total Stem Width (SW) observed between NSLM species and samples grown under lunar gravity (lun-g) and terrestrial control conditions were not as pronounced as those observed for the other parameters. Even so, it was observed that NSLM samples grown under lunar gravity (lun-g) had a stem width at least 30 per cent greater (29.12% for Alfalfa (*Medicago sativa*)) than NSLM samples in the terrestrial controls.

The highest to lowest difference in total (final day, day 4) Stem Width (SW) was found in the following order: 41.35% Mustard (*Sinapis alba*), 33.61% Broccoli (*Brassica rapa var cymosa*) and Mungbean (*Vigna radiata*), 31.96% Radish (*Raphanus sativus var sativus*), and 29.12% Alfalfa (*Medicago sativa*).

To date, no study has reported quantitative measurements or percentage changes in stem width in simulated microgravity conditions, which limits the possibility of direct comparison with the present

results. Further research is needed to investigate the morphological and physiological responses associated with variations in stem width in space environments.

4. GENERAL CONCLUSIONS

This research study presents the results of the successful cultivation of 5 (five) microgreen varieties - Broccoli (*Brassica rapa var cymosa*), Radish (*Raphanus sativus var sativus*), Mustard (*Sinapis alba*), Alfalfa (*Medicago sativa*), and Mungbean (*Vigna radiata*) - under simulated lunar gravity (lun-g) conditions using a minimalistic Nutrient-Substrate-Soil-less Microsystem (NSLM), comparing such development performance features with terrestrial controls.

The results showed that all five (5) selected microgreen varieties developed successfully in the NSLM. Impressively, the samples exposed to simulated lunar gravity (lun-g) showed accelerated growth compared to the ground controls, reaching full maturity in just four (4) days. Morphological and physiological parameters, including fresh weight, stem length, and stem width, were consistently higher for the NSLM samples under simulated lunar gravity (lun-g) compared to their respective terrestrial controls.

Future research should investigate further the present experimental setup, including - but not limited to - nutritional value, metabolic profiling, atmospheric composition, radiation, and integration with closed-loop bioregenerative cycles. Understanding these mechanisms will be essential to the sustainability of food production systems beyond Earth

Declarations

6. AUTHORS' CONTRIBUTIONS

C.N.C.S-M. conceived the study, designed the experimental setup, and performed the experiments. S.C. contributed to experimental execution and data collection. H.M. contributed to the manuscript review and editing. C.S.I. supervised the research and provided scientific guidance throughout the project. All authors reviewed and approved the final manuscript.

7. DATA AVAILABILITY

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

8. COMPETING INTERESTS STATEMENT

The authors declare no competing interests.

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Figures

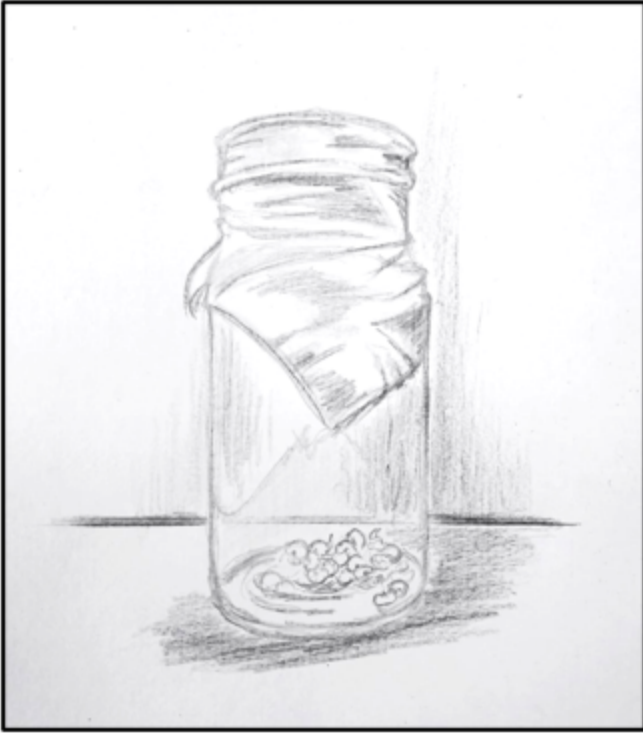


Figure 1

NSLM experimental setup.

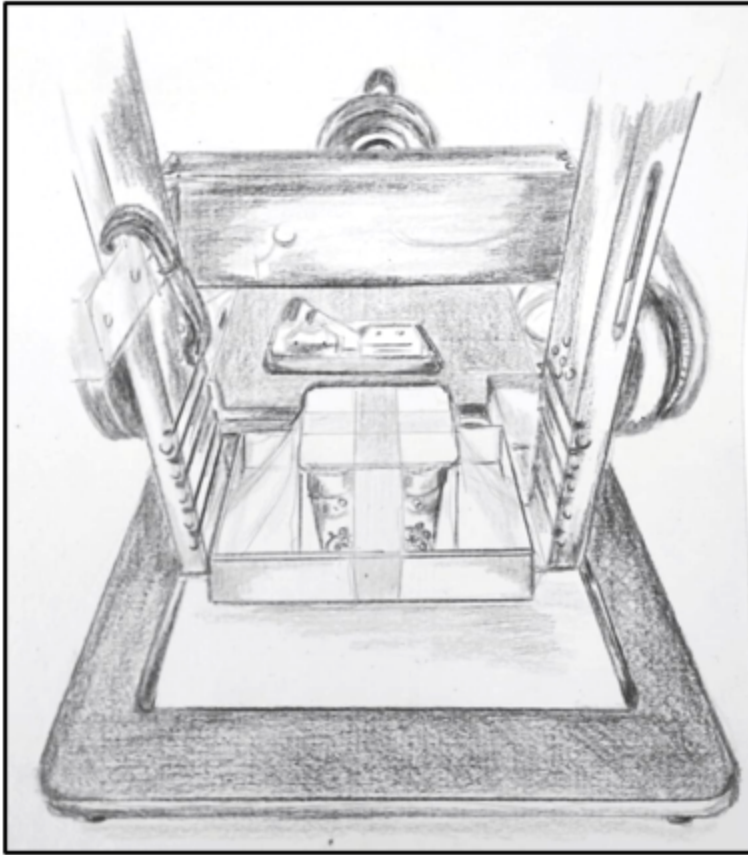


Figure 2

NSLM-RPM experimental setup.

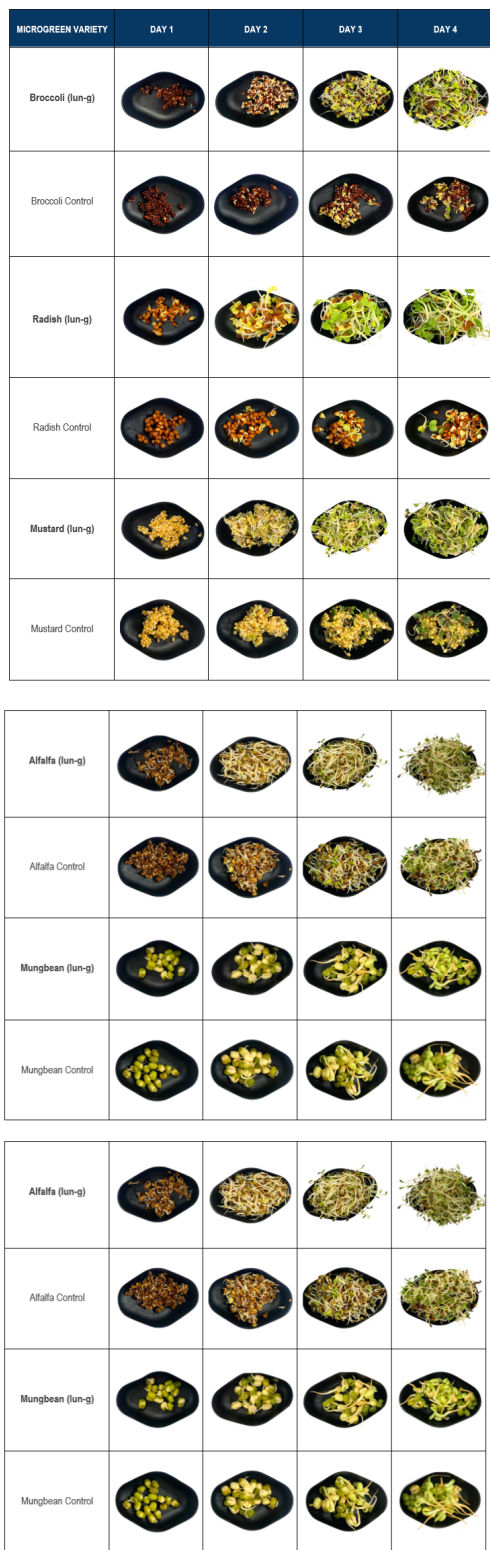


Figure 3

Photographs of the NSLM samples to compare the morphological traits for Broccoli (*Brassica rapa var cymosa*), Radish (*Raphanus sativus var sativus*), Mustard (*Sinapis alba*), Alfalfa (*Medicago sativa*), and Mungbean (*Vigna radiata*), under simulated lunar gravity (lun-g) and terrestrial control.

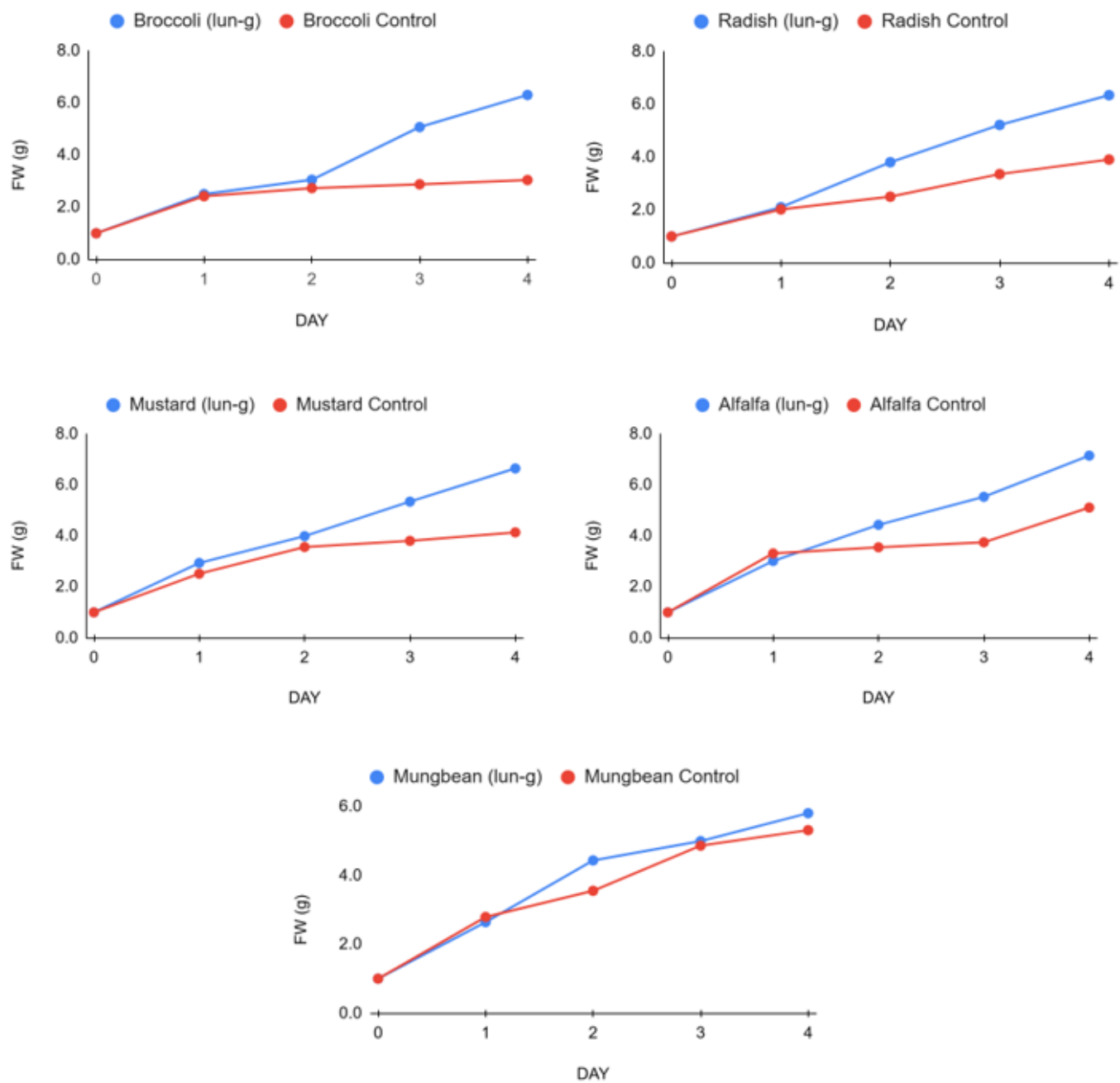


Figure 4

Fresh Weigh (FW) response for Broccoli (*Brassica rapa* var *cymosa*), Radish (*Raphanus sativus* var *sativus*), Mustard (*Sinapis alba*), Alfalfa (*Medicago sativa*), and Mungbean (*Vigna radiata*), for samples NLSM under simulated lunar gravity (lun-g) and terrestrial control.

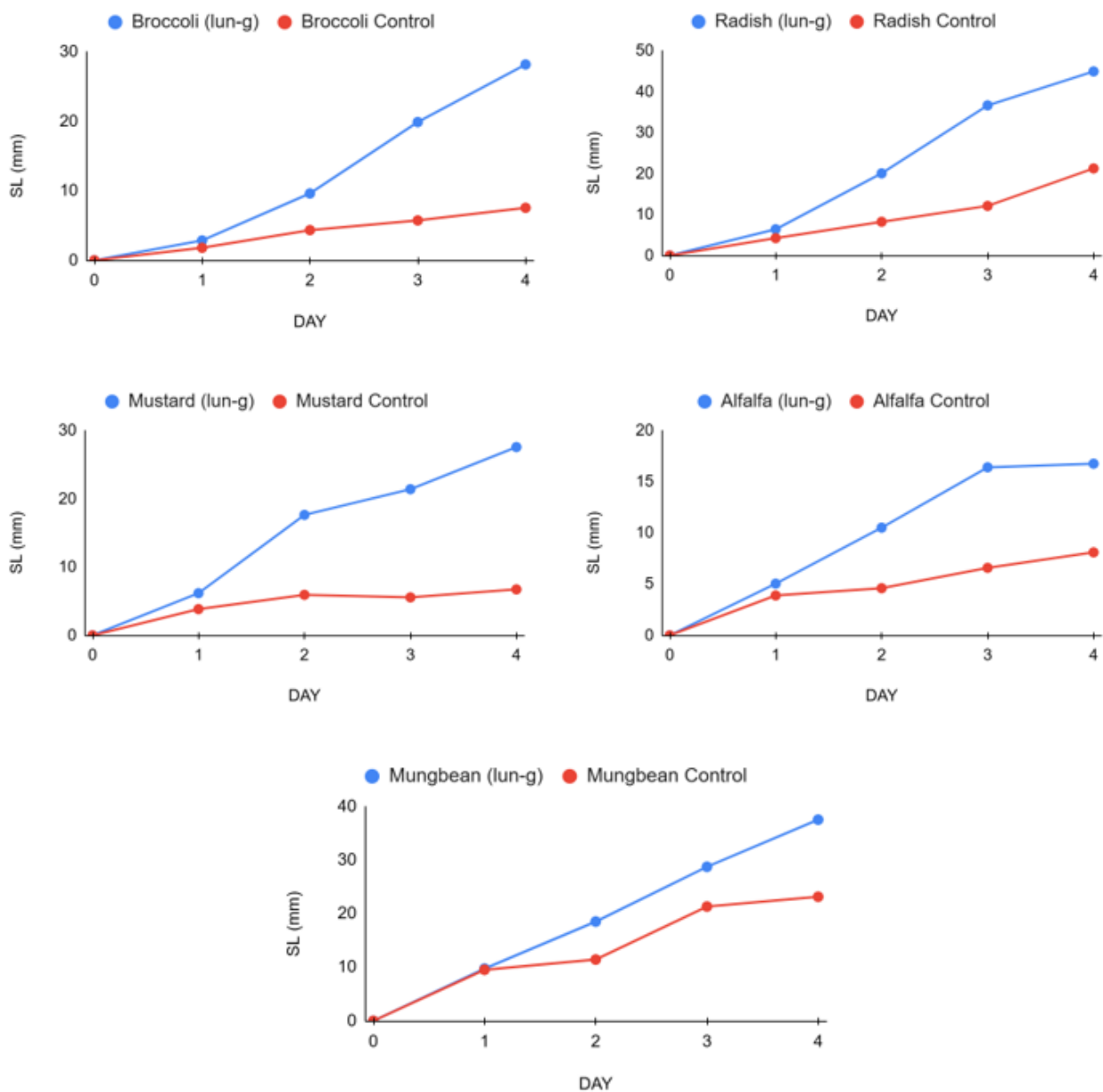


Figure 5

Stem Length (SL) response for Broccoli (*Brassica rapa var cymosa*), Radish (*Raphanus sativus var sativus*), Mustard (*Sinapis alba*), Alfalfa (*Medicago sativa*), and Mungbean (*Vigna radiata*), for samples NLSM under simulated lunar gravity (lun-g) and terrestrial control.

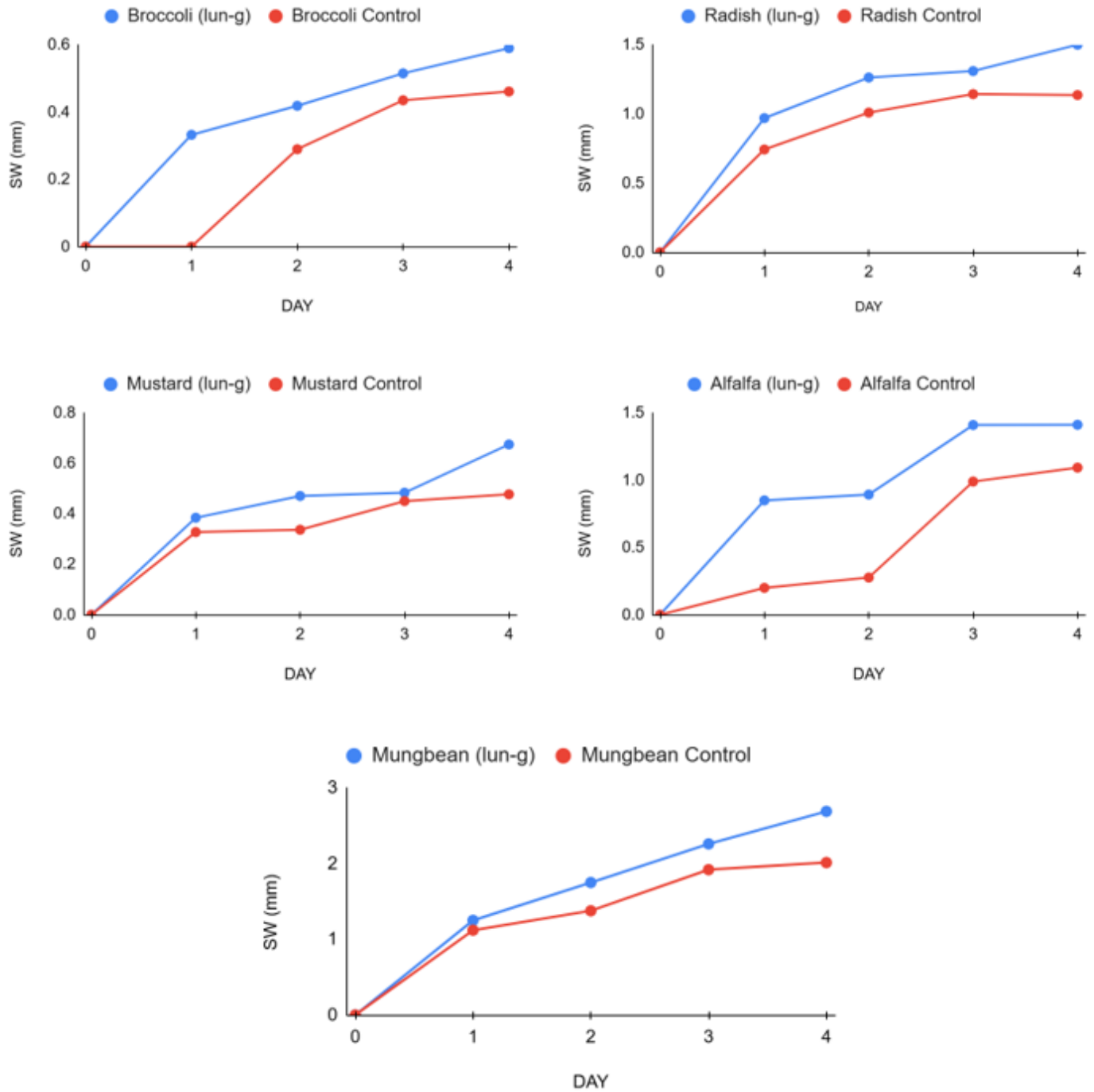


Figure 6

Stem Width (SW) response for Broccoli (*Brassica rapa* var *cymosa*), Radish (*Raphanus sativus* var *sativus*), Mustard (*Sinapis alba*), Alfalfa (*Medicago sativa*), and Mungbean (*Vigna radiata*), for samples NLSM under simulated lunar gravity (lun-g) and terrestrial control.