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Direct Contact Membrane Distillation of Artificial Urine for Sugar Beet Production in a Hydroponic System

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ABSTRACT

Appropriate nutrient sources and optimized doses of plant nutrients for space and lunar farming have remained key challenges prompting investigations to sort out biologically viable options including human urine. Therefore, a trial was performed to compare the hydroponic growth of sugar beets using a standard nutrient solution and the same nutrient solution with a 10% replacement of an artificial urine solution that had been concentrated using direct contact membrane distillation (DCMD). The response variables included yield-contributing traits and root parameters (plant height, stem diameter, length and fresh weight of leaf and root, whole plant fresh weight), along with beet characteristics (diameter, length, and fresh weight at harvest). The results revealed that 10% synthetic urine treatment produced significantly taller plants (33%, 3%, and 8% at the 4th, 6th, and 10th week after sowing, respectively) and recorded 52% and 40% greater leaf width at the 4th and 6th weeks, respectively, compared to the control. In contrast, 10% replacement with synthetic urine in the nutrient performed statistically below par compared to the control treatment by producing 9% and 17% lower leaf width at the 8th and 10th weeks, respectively. Additionally, at the harvest, 10% synthetic urine treatment gave taller plants with greater stem length and root length (2.3%, 8.6%, and 59%, respectively) than the control. Moreover, the replacement treatment remained superior by showing higher root weight and stem diameter at harvest but performed below par compared to the control in leaf width and whole plant fresh weight. At harvest, both treatments remained statistically non-significant in terms of beet length, however the control surpassed synthetic urine treatment by yielding 37% and 103% higher width and fresh weight of beets, respectively. Based on recorded findings, it may be inferred that synthetic urine holds potential as a valuable plant nutrient source for producing sugar beets in an indoor hydroponic system, though not comparable in some respects with the control (standardized plant nutrient medium) for some plant measurements.

Keywords: lunar agriculture, membrane, sugar beet, sodium chloride stress, synthetic urine, beet weight, hydroponic system, deep space.

INTRODUCTION

Recently, space farming has evolved rapidly, aiming to grow food crops in space to support long duration space missions along with colonization of other planets and Earth's moon. Researchers strive to find ways to strengthen food crop cultivation in space by improving self-sufficiency through technological advancements (Wheeler, 2017). Particularly, efficient utilization of limited resources, such as nutrient recycling, is essential in determining the production efficacy of food crops in lunar farming (Wright et al., 2023). The provision of substantial quantities of nutrient solutions to deep space missions has remained troublesome due to challenges concerning preparation, concentrating the nutrient solutions, and fuel-intensive transportation. Therefore, optimizing on-site available, abundant, and renewable plant nutrient resources has become strategically pivotal for lunar farming (Maury et al., 2020).

The human kidney excretes urine that contains plant nutrients. Urine contains nutrients released from human food that are not consumed in energy metabolism and new cell growth (Simha et al., 2024). A wide array of factors, such as time of urination, diet, physical health of a human, climate, body size, and physical activity level determine the chemical composition of human urine (Rose et al., 2015). Among chemical compounds of human urine, urea predominates along with other macronutrients (phosphorus and potassium) and secondary nutrients (e.g., calcium, magnesium, and iron) making urine qualified as a valuable plant nutrient source (Simha et al., 2023). Although urine has been revealed to be extremely complex, its organic metabolites primarily include urea, creatinine, hippuric acid, and citric acid (Zhang et al., 2021). The availability of urine in human-crewed space missions also favors its utilization as a valuable source of water and plant nutrients for crop production

(Karak and Bhattacharyya, 2011). Lunar farming requires the cultivation of crops that are not only low in resource acquisition and high in resource use efficiency, but also have a short crop growth cycle. Sugar beets (Beta vulgaris L.) are believed to have originated in North Africa and some European regions, and they have become a vital sugar crop as well as a vegetable (Iqbal and Saleem, 2015). They have emerged as the second largest raw material of refined table sugar accounting for over 30-40% of sugar production globally, and their by-products find their use in preparing biofertilizers, bioethanol, food additives, and a variety of biodegradable polymers (Zhang et al., 2016). They have a wide adaptability to an extensive range of climatic conditions, high nutritional quality (Tyburski et al., 2024), and a short life cycle of 90-120 days (Iqbal et al., 2015), which makes them suitable for hydroponic and aeroponic cultivation. Previously, it has been reported that yield and nutritional quality traits of hydroponically grown lettuce cultivated with enriched urine were comparable to those obtained with a commercial fertilizer (Jurga et al., 2021). Moreover, it has been inferred that the optimum yield of hydroponically grown cabbage can be obtained from urine and water application in a 1:3 ratio (Alemayehu et al., 2020). Furthermore, beet plants' high salt tolerance (40-120 mM of NaCl) (Subbarao et al., 2001; Yang et al., 2012), make them one of the most appropriate choices for hydroponic production receiving urine as a source of plant nutrients.

However, research gaps exist pertaining to the concentration and utilization of urine (human and synthetic) for sugar beet production in an indoor hydroponic system. This is because previous research findings are scant and report contradictory conclusions, necessitating fresh studies. Thus, we hypothesized that synthetic urine in optimized quantities might be utilized as a plant nutrient source owing to the presence of all macronutrients (nitrogen, phosphorous, and potassium) for achieving a high yield of hydroponically grown sugar beets. Therefore, the prime aim of this investigation was to comparatively evaluate the performance of synthetic urine and the control treatment (entailing standardized plant nutrients growth medium) in terms of yield contributing traits, along with the root and beet parameters of sugar beets grown in an indoor hydroponic system.

MATERIALS AND METHODS

The trial was conducted in the biomass lab of the Department of Chemical Engineering, Louisiana Tech University, Ruston, LA, United States during 2024. Two 6.5-liter hydroponic pod kit systems (Uruq, HPS-Shenzhen Huijujiapin Co. Ltd., China), each capable of growing beet plants were purchased for executing the hydroponic trial. A standard nutrient medium (Aero Garden, Aero Grow Industries, USA), containing nitrogen (4%), phosphorus (3%), potassium (6%), calcium (1%), and magnesium (0.9%), was used as a control treatment for comparison with partial synthetic urine in an indoor hydroponic system. Sugar beet seeds were sourced from Johnny Seeds Ltd. (Maine, USA) for cultivation in the hydroponic system.

Preparation of artificial urine

Human urine comprises over 3.000 components, representing individuals of all ages, races, genders, and regions. Among these, more than 90 compounds are consistently found in every sample, regardless of gender or the time of collection (Bouatra et al., 2013). For practicality and cost-effectiveness, the formulation of artificial urine has been simplified by including only 13 components (Sarigul et al., 2019). In this study, artificial urine was prepared by dissolving specified amounts of thirteen chemicals (Table 1) in 1000 ml of deionized (DI) water. The solution was placed in a 2000 ml flask on a hot plate with a magnetic stirrer (200 rpm at room temperature). Each compound was added sequentially, to ensure complete dissolution in the deionized water. After all components were added, the mixture was stirred for 1 hour to achieve a clear, homogeneous solution. The solution was sealed and stored at room temperature preceding the direct contact membrane distillation (DCMD) process.

Chemical name	Chemical formula	Quantity (gram)	Supplier
Sodium sulfate	Na ₂ SO ₄	1.700	Flinn Scientific, Batavia, IL, USA
Uric acid	C ₅ H ₄ N ₄ O ₃	0.250	Sigma Aldrich, Switzerland
Sodium citrate	Na ₃ C ₆ H ₅ O ₇ ·2H ₂ O	0.720	Flinn Scientific, Batavia, IL, USA
Creatinine	C ₄ H ₇ N ₃ O	0.881	Sigma Aldrich, St. Louis, MO, USA
Urea	CH ₄ N ₂ O	15.000	Sigma Aldrich, St. Louis, MO, USA
Potassium chloride	KCI	2.308	Flinn Scientific, Batavia, IL, USA
Sodium chloride	NaCl	1.756	Fisher Scientific, Fair Lawn, NJ, USA
Calcium chloride	CaCl ₂	0.1850	Flinn Scientific, Batavia, IL, USA
Ammonium chloride	NH ₄ CI	1.266	Flinn Scientific, Batavia, IL, USA
Potassium oxalate monohydrate	$K_2C_2O_4 \cdot H_2O$	0.035	Flinn Scientific, Batavia, IL, USA
Magnesium sulphate	MgSO ₄ ·7H ₂ O	1.082	Flinn Scientific, Batavia, IL, USA
Sodium phosphate dihydrate	NaH ₂ PO ₄ ·2H ₂ O	2.912	Aldon Corporation, NY, USA
Sodium phosphate dibasic dihydrate	Na ₂ HPO ₄ ·2H ₂ O	0.831	Sigma Aldrich, St. Louis, MO, USA

Table 1. Chemical compounds, their chemical formulas	s, quantities used and manufacturer for the preparation of
synthetic urine	

Direct contact membrane distillation of artificial urine

DCMD has been regarded as an energy-efficient thermal separation process that uses a hydrophobic, microporous membrane to separate a hot feed solution from cold water. The process relies on the temperature difference across the membrane, which creates a vapor pressure gradient, allowing water vapor to transfer from the hot feed side to the cold-water side. The hot feed urine solution comes into direct contact with cold DI water on the opposite side of the membrane. The hydrophobic membrane permits only water vapor to pass through its pores, retaining the liquid phase and larger or ionic dissolved substances. The DCMD operates at relatively low temperatures (50 °C, 65 °C, and 80 °C) and pressures compared to traditional desalination or wastewater treatment methods, offering simplicity and potentially lower implementation costs. In this study, a 1-liter flask containing artificial urine was placed in a hot water bath system (model 1130A, VWR International, Radnor, PA, USA) and maintained at constant temperatures of 50 °C, 65 °C, or 80 °C for the three different experimental settings. Meanwhile, another 1-liter flask containing 200 ml of DI water was kept close to 0 °C using a chiller (model 9510, PolyScience, Niles, Illinois, USA). A PTFE membrane (145×97 mm flat sheet membrane of 0.45 microns pore size, STERLITECH, Auburn, WA, USA) was used to perform the DCMD process. Peristaltic pumps

(model 77200-50, Masterflex, Vernon, IL, USA) were used for fluid circulation. The process was performed for 8 hours, with 50 mL samples collected from the hot side every 2 hours to assess membrane effectiveness and observe changes. Analyses of membrane flux, density, ionic conductivity, pH, and energy consumption were performed to ensure accurate evaluation of the changes resulting from DCMD treatment. The initial concentration of substances, including all chemical compounds, was 28,000 ppm. After 8 h of DCMD operation, the concentration increased to 34,000 ppm, 54,000 ppm, and 84,000 ppm at temperatures of 50 °C, 65 °C, and 80 °C, respectively. The 84,000 ppm concentrated synthetic urine was used to replace 10% of the standard nutrient solution for the experiments.

Set-up of the hydroponic system for sugar beet production

Two hydroponic pod systems were placed under a fume hood to maintain the controlled light, temperature, and airflow conditions. The temperature was kept constant at 25 °C, with the built-in light on for 16 hours daily (5:00 AM to 9:00 PM) and off for 8 hours (9:00 PM to 5:00 AM) using a built-in controller. One pod served as a control with only the standard nutrient feed, while the other pod was used for plant growth in a 10% 84,000 ppm urine stream, supplemented with a 90% standard nutrient solution. Before placement in the pod system, sugar beet seeds were submerged in deionized (DI) water for 8 hours to enhance germination. The 6.5-liter hydroponic pod trays were filled with 5 liters of DI water, and 24 ml of standard nutrient medium was added to each tray as the initial treatment. After 8 hours of soaking, three seeds were placed into each sponge, which were then inserted into the pod system. Germination began three days after placement and completed within one week. Only the dominant plant from each germinated group was retained for growth, while others were removed. A small built-in pump in the hydroponic kit system operated on a 30-minute on-off cycle to oxygenate the root system, and the fume hood window and exhaust fan were kept open for airflow. From the second treatment onwards, the control plants received standard nutrient solution, while the plants in the urine stream received a mix of standard nutrient solution with 10% 84,000 ppm urine solution, both applied at two-week intervals. The treatment continued until the eighth week, and the plants were harvested in the tenth week. Throughout the growing period, DI water was added weekly to each tray as needed to maintain a water level of 5 liters, and the amount added was recorded by mass.

Recording of plant growth response variables

During plant growth, the width and length of each leaf, as well as the overall height of each plant, were measured and recorded using a measuring tape. This measurement process began in the fourth week, once 3–5 leaves had developed on each plant, and continued weekly until the tenth week, when the plants were harvested. At harvest, the width and length of the plant leaves, root length, beet length, and overall plant height were recorded. Additionally, stem and beet diameters at three positions (top, middle, and bottom) were measured using digital calipers. The roots of each plant were patted dry with a paper towel before their weight was recorded. Plant weight and beet weight of each plant were measured using a balance.

Statistical analysis of the recorded data

The recorded data of sugar beets were arranged and subjected to statistical analysis by performing one-way analysis of variance (ANOVA) as per completely randomized design (CRD) to estimate the overall significance of the employed treatments using the computer-run statistical package (Statistix version 10.0). Subsequently, the least significant different (LSD) test at 5% probability level was put into practice to determine the significance among treatment means by following the procedure laid down by Gomez and Gomez (1984).

RESULTS AND DISCUSSION

Plant height and leaf width at two-week intervals

The recorded data pertaining to the plant height and leaf width of hydroponically grown sugar beets explored the pronounced influence of employed treatments on these response variables at all intervals under investigation (Figures 1a and 1b). The results concerning the plant height of sugar beets at the fourth week after sowing revealed that synthetic urine replacement remained superior by recording 30% taller plants than the control treatment (Figure 1a). Likewise, the control treatment produced 3% shorter sugar beet plants in comparison to the 10% synthetic urine treatment at six weeks, but both treatments performed statistically at par with each other at the eighth week of the trial. However, 10% synthetic urine gave 8% taller sugar beet plants than the control treatment at the 10th week of the trail. Concerning the leaf width of sugar beets grown in a hydroponic system, the results depicted in Figure 1b show a statistically significant effect on the leaf width at all intervals under investigation (Figure 1b). It was noted that synthetic urine (10% replacement) surpassed the control treatment by a 52% and 40% greater leaf width at the fourth and sixth weeks, respectively. However, a deviation from this trend was recorded for the leaf width of sugar beets at eight and ten weeks where 10% synthetic urine performed statistically below par compared to the control treatment by producing 9% and 17% lower leaf width at the 6th and 8th weeks, respectively. The recorded findings were in concurrence with the postulated research hypothesis as the employed treatments (10% synthetic urine and control treatment versus a standardized nutrient medium) performed differently for all response variables including plant height and leaf width of the sugar beets grown in an indoor hydroponic system. As per the recorded findings of this study, synthetic urine remained superior initially by producing significantly taller plants with a greater leaf width, however at later growth stages of the crop, the control treatment surpassed the 10% synthetic

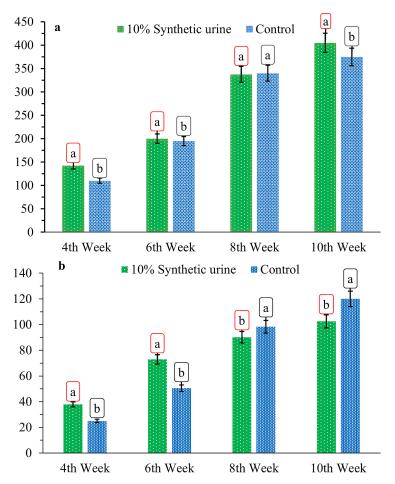


Figure 1. Comparative assessment of synthetic urine (10% replacement) and control treatment (standardized plant nutrient medium) for (a) plant height (from base of the stem to the tip of the leaf) (mm) and (b) leaf width (mm) of sugar beet grown in a hydroponic system. Different letters indicate significant differences among treatment means at the 5% probability level

urine treatment. These results may be attributed to the abundant provision of macronutrients, particularly nitrogen that triggered the plant growth and ultimately the plant height and leaf width were significantly improved. Likewise, the suboptimal performance of synthetic urine in terms of plant height and leaf width at later growth stages (at the 8th and 10th weeks of the trial) could be linked to the higher uptake of sodium chloride that hampered the vegetative growth of sugar beets. These findings were in line with the previous studies whereby urine application was considered a biologically viable option to furnish primary nutrients for crop production (Pradhan et al., 2007), and it was suggested that human urine could be utilized to supply essential nutrients in an aquaculture approach through a constructed food chain (Adamsson, 2000). However, higher accumulation of NaCl in plants has been reported to seriously hamper the vegetative growth of many crops, such as mung bean (Shaddam et al., 2024) and maize (Islam et al., 2024).

Plant height, stem length, and root length at harvest

The results pertaining to plant height, stem, and root lengths of hydroponically grown sugar beet at the time of harvest indicated a strong influence of employed treatments (Figure 2). The employed treatment of 10% synthetic urine replacement recorded significantly taller sugar beet plants (2.3% higher than the control treatment). Likewise, the sugar beet stem lengths was also influenced by the employed treatments as 10% synthetic urine increased the stem length (8.6% greater than the corresponding value recorded by the control). Moreover, the results on the root length of sugar beets exhibited that synthetic urine (10%) remained effective in boosting the root length by 59% compared to the control treatment. These findings corroborate those of previous studies where it was inferred that urine contained macronutrients (N, P, and K) that assisted crop plants to attain vigorous vegetative growth; particularly N was involved

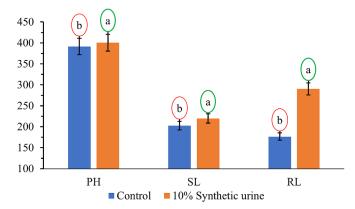


Figure 2. Comparative assessment of synthetic urine (10% replacement) and control treatment (standardized plant nutrient medium) for plant height (PH, from the base of the stem to the tip of the leaf) (cm), stem length (SL, mm), and root length (RL, mm) at the harvesting time of sugar beets grown in a hydroponic system. Different letters indicate significant differences among treatment means at the 5% probability level

in producing significantly taller wheat plants (Kizilgeci et al., 2021). Moreover, human urine $(20000 \text{ L}\cdot\text{ha}^{-1})$ performed better compared to the application of 400 kg·ha⁻¹·NPK (15:15:15) chemical fertilizer, giving a pronouncedly higher yield of okra (Akpan-Idiok et al., 2012) and other vegetables (Mnkeni et al., 2008). Furthermore, similar to our findings, sufficient P availability has been reported to impart a positive influence on the root development of crops (Paul et al., 2023).

Root weight and stem diameter at harvest

At harvest, the recorded findings revealed that synthetic urine and control treatments imparted a statistically pronounced influence on the response variables (root weight and stem diameter) of hydroponically grown sugar beets (Figure 3). As far as the root weights of sugar beets were concerned, the application of synthetic urine (10%) exhibited a 26% higher root weight in comparison to the control treatment. In contrast, the control treatment outperformed the synthetic urine in terms of stem diameter by recording a 5% greater value compared to the synthetic urine application. Greater root development may be a response to the presence of higher salt content of urine, enabling the plants to get rid of excessive salts. It has been reported that P supplied by a source (synthetic urine in this study) is effective in triggering the growth of beet plants root network. However, it was also inferred that a greater concentration of salts (particularly NaCl in synthetic urine) significantly reduced the yield attributes of vegetables through osmotic stress, which led to reduced uptake of water and ionic imbalance in sugar beets (Barbosa et al., 2024). Furthermore, previous research findings have reported a 10% increase in root biomass of red beets owing to the optimized application of urine (Pradhan et al., 2010). Our findings contradict the findings of Li et al. (2018), who opined those low concentrations of micronutrients in growth media triggered

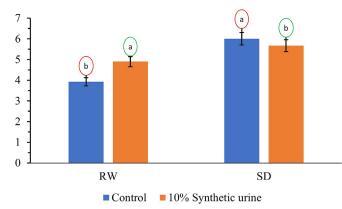


Figure 3. Comparative assessment of synthetic urine (10%) and control treatment (standardized plant nutrient medium) for root weight (RW, g) and stem diameter (SD, mm) at harvest of sugar beets grown in a hydroponic system. Different letters indicate significant differences among treatment means at the 5% probability level

root development at the expense of shoot growth for hydroponically grown lettuce.

Leaf length, fresh leaf weight, and fresh whole plant weight at harvest

In this study, the recorded data revealed that the fresh weight of leaf, along with the whole plant's fresh weight at the time of harvest of hydroponically grown sugar beets were greatly influenced by the employed treatments (Figure 4). Concerning the sugar beet leaf length grown in the hydroponic systems, the control treatment (195.5 mm) performed statistically at par with the synthetic urine (10%) application (194 mm). However, 10% synthetic urine application could not match the control treatment in terms of leaf and whole plant fresh weights because the control treatment gave 16% and 24% higher leaf fresh weight and whole plant fresh weight, respectively, compared to synthetic urine. Based on the recorded findings of this study, it might be inferred that salt accumulation in the leaves hampered the sugar beets vegetative growth (leaf length and width along with fresh weight of the whole plants), whereas absence of NaCl in the control treatment resulted in larger leaf growth. Previously, it was reported that pure urine produced similar growth and yield for cucumbers as that of commercial fertilizer (Heinonen-Tanski et al., 2007), however this finding could be attributed to the varying composition of urine (not as concentrated) and to the different crop species.

Diameter, length, and fresh weight of beets at harvest

The recorded findings exhibited that synthetic urine (10%) performed below par statistically with the control treatment concerning the beet response variables (diameter, length, and

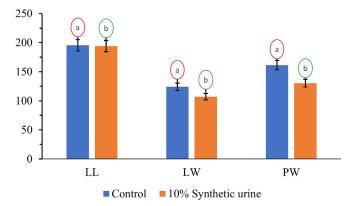


Figure 4. Comparative assessment of synthetic urine (10%) and control treatment (standardized plant nutrient medium) for leave length (LL, mm), leaf weight (LW, g), and plant weight (PW, g) at the harvesting time of sugar beets grown in hydroponic system. Different letters indicate significant differences among treatment means at the 5% probability level

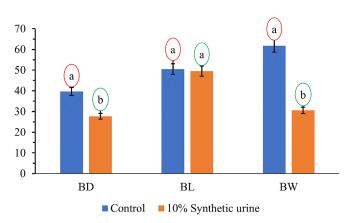


Figure 5. Comparative assessment of synthetic urine (10%) and control treatment (standardized plant nutrient medium) for beet diameter (BD, mm), beet length (BL, mm), and beet fresh weight (BW, g) at the harvesting time of sugar beets grown in a hydroponic system. Different letters indicate significant differences among treatment means at a 5% probability level

fresh weight of beets) at the sugar beet harvesting time after growth in an indoor hydroponic system (Figure 5). The recorded findings demonstrated that the application of synthetic urine resulted in a net reduction of 37% in beet diameter compared to the control treatment. However, 10% synthetic urine and control treatments remained statistically at par with each other in terms of beet length with beet lengths of 50.5 mm and 49.5 mm, respectively. Interestingly, the control treatment remained superior to the synthetic urine by recording 103% greater fresh weight of beets. These results could be attributed to significantly lesser leaf length and width along with the fresh weight of the whole plant produced with synthetic urine replacement, which ultimately led to the suboptimal beet characteristics. These findings contradict those of Pradhan et al. (2010), who inferred that the optimum concentrations of macronutrients (N, P, and K) along with secondary nutrients (particularly calcium and magnesium) in human urine remained effective in boosting the beet growth (length, diameter, fresh weight, etc.) of red beets. Moreover, secondary nutrients (Ca and Mg) supplied by urine were also reported to positively influence the yield of cabbage (Pradhan et al., 2007).

CONCLUSIONS

The recorded findings of this trial were in line with the postulated research hypothesis as a 10% replacement of standard nutrient medium with DCDM-concentrated synthetic urine demonstrated its efficacy as a valuable plant nutrient source owing to the presence of all macronutrients (nitrogen, phosphorous, and potassium) for achieving a yield of hydroponically grown sugar beets. The 10% synthetic urine medium was superior to the control treatment in terms of plant height, stem diameter, leaf width, stem length, and root length, while it performed at par with the control for the leaf length and beet length. However, it remained inferior to the control treatment for the leaf width and whole plant fresh weight along with diameter and fresh weight of beets probably due to the accumulation of sodium chloride and slight ammonia toxicity from urea conversion to ammonia. Despite this, 10% concentrated synthetic urine demonstrated its potential and efficacy as a valuable plant nutrient source for supplying essential nutrients (macro and secondary nutrients) for sugar beet production in an indoor hydroponic system. However, future

research needs to focus on a comparative assessment of other concentrations of synthetic urine to optimize its dose for hydroponically grown sugar beet, lettuce, and cabbage crops with the broader perspectives of utilizing research findings in lunar and space farming.

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