

Original Scientific Paper

Cultivating watermelon (*Citrullus lanatus*) in Martian regolith simulant after seed inoculation with plant growth-promoting bacteria

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ABSTRACT:

The human colonisation of space is no longer confined to the realm of science fiction. Among various celestial bodies, Mars has emerged as a primary target for long-term human settlement in the future. One of the major challenges in such missions is ensuring a sustainable food supply. Transporting food across such vast distances is both costly and impractical, prompting growing interest in space farming and the use of *in situ* resources, such as Martian regolith, for plant cultivation. In this study, we explored the potential of growing watermelon (*Citrullus lanatus*) under simulated Martian conditions using a substrate which mimics the composition of Martian regolith. To mitigate nutrient deficiency, the seeds were treated with plant growth-promoting bacteria (PGPB) isolated from the rhizosphere of *Miscanthus × giganteus* grown in heavy metal-contaminated soil, yielding bacterial strains tolerant to the metal-rich and nutrient-poor conditions analogous to those of the Martian regolith simulant. Statistically significant differences in the plant growth parameters - including height, root length, fresh weight, and leaf area - were observed between the plants grown on soil and regolith substrates, while the chlorophyll content showed no significant variation, suggesting a preserved photosynthetic function despite abiotic stress. Additionally, seed inoculation with a bacterial consortium consisting of *Pseudomonas chlororaphis*, *Bacillus safensis*, and *B. cereus/thuringiensis* improved the germination rates compared to the untreated control. This research represents the first attempt to cultivate watermelon in a Martian regolith simulant and highlights the potential of PGPB as a promising strategy to enhance plant performance under extreme conditions. Further studies are needed to optimise microbial consortia and regolith amendments for future space agriculture applications.

Keywords: BLSS, ISRU, microbial consortia, space agriculture, space colonisation

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INTRODUCTION

Since ancient times, humanity has been driven by a deep curiosity to understand the cosmos and explore the potential for life beyond Earth. This quest has gradually evolved from philosophical speculation into scientific investigation. Among all the celestial bodies in our solar system, Mars has emerged as a particularly compelling target - due not only to its proximity and surface features, but also to mounting evidence that it may once have harboured conditions suitable for life (BEECH & COMTE 2021). Although Mars is now a cold, dusty desert with a thin atmosphere, it remains the leading candidate for future human exploration and long-term settlement (GILLMANN *et al.* 2009; MAITY & SAXENA 2024).



In all scenarios involving long-term human presence on Mars, plants are considered essential components of a bioregenerative life support system (BLSS) (KANE & SHASTEEN 2024). A fully functional BLSS must be capable of recycling water, regenerating the atmosphere, and sustaining food production within a closed-loop system (DURI *et al.* 2022). Plants play a central role by converting carbon dioxide into oxygen and food, while simultaneously processing human waste into usable nutrients, thus forming a self-sustaining cycle crucial to both space habitats and Earth ecosystems (FERL *et al.* 2002). To reduce dependency on supplies from Earth and enhance sustainability, native regoliths are being evaluated as potential substrates for plant cultivation (EICHLER *et al.* 2021). Integrating *in situ* resource utilisation (ISRU) with BLSS is viewed as a promising strategy to enable long-term food production in off-Earth regolith (DURI *et al.* 2022).

A Martian regolith is a fine-grained, loose mixture of rocky soil and dust which lacks organic matter, contains toxic compounds such as perchlorates, and presents physical and chemical challenges to plant growth (BISHOP *et al.* 2002; RAMÍREZ *et al.* 2019). It is primarily composed of basaltic sand rich in plagioclase, olivine, and pyroxene, with smaller amounts of smectite and saponite clays, sulphate salts such as gypsum and anhydrite, and iron oxides like magnetite, hematite, and ferrihydrite, which are responsible for Mars' distinctive reddish appearance (MCSWEEN & KEIL 2000; PETERS *et al.* 2008). Based on data from the Rover, Lander, and Orbiter missions, a number of regolith simulants have been developed to support Earth-based experiments (LONG-FOX & BRITT 2023). However, given the diversity of Mars' surface, no single simulant can fully represent the planet's complexity to support life such as plants.

Plant growth trials have shown that these simulants can support short-term cultivation and provide essential minerals such as potassium, calcium, magnesium, and iron (DURI *et al.* 2022). However, they are deficient in organic matter and key macronutrients like nitrogen, phosphorus, and sulphur. Furthermore, factors such as high alkalinity, elevated sodium levels, low cohesion, poor water retention, and occasional perchlorate toxicity limit their agricultural potential (DURI *et al.* 2022).

To improve the growth potential of regolith simulants, several strategies have been proposed. These include the addition of stable organic amendments to enhance nutrient content and structure, as well as soil tillage methods to reduce water leaching in a reduced-gravity environment (MAGGI & PALLUD 2010; EICHLER *et al.* 2021). Nodulating plants and their nitrogen-fixing symbionts have also been used to enhance the fertility of Martian regolith (HARRIS *et al.* 2021). In addition, microbial consortia can be integrated to enhance nutrient availability, further supporting ISRU-based approaches (HANDY *et al.* 2021).

On Earth, plant growth-promoting bacteria (PGPB) play a crucial role in supporting plant development by producing growth hormones, enhancing nutrient uptake, fixing atmospheric nitrogen, and offering protection against pathogens (GLICK 2012). In extraterrestrial agriculture, these microbes may serve similar functions, although this remains an underexplored area with considerable potential. Their use could reduce the need for external fertilisers and improve plant resilience in closed environments (HANDY *et al.* 2021).

Previous studies have evaluated a diverse array of crop species in Martian regolith simulants - including cereals, legumes, root and bulb vegetables, solanaceous crops, and even ornamental species (DURI *et al.* 2022). The objective of this study was to evaluate the potential for cultivating watermelon (*Citrullus lanatus*) in a Martian regolith simulant, as, to our knowledge, this crop has not yet been studied under such conditions despite its potential as a valuable food and hydration source for future human missions. Watermelon originates

from Africa and, in some arid regions, has traditionally been used as a valuable water source due to its high fruit water content (about 93%) (WEHNER 2008). It is capable of achieving high water productivity under conditions of severe water deficit, indicating its capacity to convert limited water into fruit even under minimal irrigation efficiently (SINGH *et al.* 2021). Moreover, watermelon is a nutrient-rich crop, known for its high content of antioxidants including lycopene, vitamin C, β -carotene, and polyphenols, which offer anti-inflammatory and anticancer benefits, and support overall human health (ΜΑΟΤΟ *et al.* 2019). As regolith is an extremely stressful environment for Earth plants, we examined whether PGPB originating from the roots of *Miscanthus × giganteus*, a plant adapted to similar nutrient-poor, metal-contaminated soils (RAKIĆ *et al.* 2021; PEŠIĆ *et al.* 2024), could enhance watermelon growth under such conditions.

MATERIAL AND METHODS

Bacterial strains. The bacterial strains used in this study were *Pseudomonas chlororaphis* Bo, *Bacillus safensis* Do, and *Bacillus cereus/thuringiensis* F4, obtained from the microbial collection of the Microorganism-Host Interactions Group, Faculty of Biology, University of Belgrade. The strains were isolated from the rhizosphere of *Miscanthus × giganteus* plants which had been growing for two years in the flotation tailings of the Rudnik mine in Central Serbia, a substrate characterised by high levels of metal contamination and severe macronutrient deficiencies (RAKIĆ *et al.* 2021). For inoculum preparation, the strains were grown overnight in Luria-Bertani broth (10 g of tryptone (TM Media, India), 5 g of yeast extract (TM Media, India) and 5 g of NaCl (HiMedia Laboratories, India) per litre of distilled water) at 30°C with continuous shaking at 180 rpm.

Regolith simulant preparation. The Martian regolith simulant used in this study was prepared from individual mineral components. The mixture was based on the most abundant oxides commonly reported in Martian simulants (DURI *et al.* 2022). The final mixture consisted of 54% SiO₂, 18% Fe₂O₃, 10% Al₂O₃, 8% CaO, and 10% MgO. To achieve a clay-like consistency, approximately 3 litres of distilled water were added to 1 kg of the dry mixture. To improve substrate porosity, thoroughly washed quartz sand with a grain size of 1–2 mm was mixed into the substrate at a 1:1 ratio. No sterilisation was performed after regolith preparation.

Regolith simulant and soil chemical properties determination. The chemical properties of the commercial soil substrate (Grow Mix, Plagron, Netherlands), used for cultivating the control plants, and of the regolith simulant samples were determined by analysing the readily available (bioavailable) fractions of selected macroelements and nutrients. Magnesium, calcium, potassium, and phosphorus were quantified using inductively coupled plasma optical emission spectrometry (ICP-OES). The total nitrogen content was determined in accordance with the Kjeldahl procedure (SÁEZ-PLAZA *et al.* 2013), while the content of organic carbon was measured using the Tyurin method (SHAMRIKOVA *et al.* 2022).

Watermelon seeds treatment. The watermelon variety used in this study was Crimson Sweet. The seeds were washed five times with sterile distilled water and subsequently treated with bacterial suspensions. The suspensions were prepared from overnight cultures of strains Bo, Do, and F4. Each culture was centrifuged (MIKRO 220, Hettich, Germany) at 5000 rpm for 10 minutes, the supernatant was discarded, and the resulting pellet was resuspended in an

equal volume of sterile distilled water. Seed treatments were performed by incubating the seeds for 10 minutes in a suspension of strain F4 alone (10 mL), a combination of strains F4 and Do (5 mL each), and a combination of all three strains Bo, Do, and F4 (3.33 mL each). These combinations were selected based on preliminary screening in which the individual strains and their mixtures were evaluated for their effects on watermelon growth in regolith-like conditions (unpublished data). The seeds treated with sterile distilled water were used as the negative control. After treatment, the seeds were air-dried in a laminar flow hood for one hour prior to planting.

Plant cultivation and growth monitoring. The watermelon seeds treated with PGPB or sterile distilled water were sown in 14 replicates in parallel in two different substrates, the Martian regolith simulant and a commercial soil substrate. The plants were grown in a growth box (Hydro Shoot HS60, Secret Jardin, Belgium) at room temperature under a 14/10 h light/dark photoperiod. They were watered every two days with approximately 10 mL of tap water. Seed germination was evaluated by calculating the germination rate, defined as the percentage of seeds which successfully germinated within the observation period. After four weeks, the following parameters were measured: the plant height, root length, fresh weight, the number of leaves, total leaf area per plant, and the chlorophyll content. The total leaf area was calculated using ImageJ software (SCHNEIDER *et al.* 2012). For the chlorophyll quantification, 0.02 g of leaf tissue from each plant sample was immersed in 2 mL of 96% ethanol and incubated at 70°C for 10 minutes. Following incubation, the absorbance of the extract was measured at wavelengths of 648 nm and 664 nm using a spectrophotometer (SPECTROstar Nano, BMG Labtech, Germany). The obtained absorbance values were applied to standard equations to calculate the concentrations of total chlorophyll (Chl total), chlorophyll a (ChlA), and chlorophyll b (ChlB) (RITCHIE 2008):

$$\begin{aligned}\text{Chl total} &= 5.24 \times A_{664} + 22.24 \times A_{648} \\ \text{ChlA} &= 13.36 \times A_{664} - 5.19 \times A_{648} \\ \text{ChlB} &= 27.43 \times A_{648} - 8.12 \times A_{664}\end{aligned}$$

In the formula, A_{664} and A_{648} denote the absorbance of the sample at 664 nm and 648 nm, used for chlorophyll determination.

Statistical analyses. Statistical analyses were performed in RStudio (Version 4.5.1) using R software (R CORE TEAM 2024). The data distribution was assessed using the Shapiro-Wilk test (`shapiro.test` function from base R) applied to both raw and log-transformed values. Since several datasets showed deviations from normality, non-parametric statistical approaches were also used. To test the effect of the treatments and substrates on the measured plant parameters, we used Permutational Multivariate Analysis of Variance (PERMANOVA) via the `adonis2` function from the `vegan` package (OKSANEN *et al.* 2022). This method is suitable for data which do not meet the assumptions of parametric ANOVA. Statistical significance was assessed at an alpha level of 0.05. For post-hoc comparisons between the treatment groups and substrates, we applied a pairwise permutational test using the `pairwise.adonis2` function from the `pairwiseAdonis` package (MARTINEZ ARBIZU 2020). Descriptive statistics (mean and standard deviation) were calculated using the `dplyr` package (WICKHAM *et al.* 2023), while data visualisations were performed using `ggplot2` (WICKHAM 2016).

Table 1. Chemical properties of the commercial soil and Martian regolith simulant substrates.

Analyzed parameter	Unit	Bioavailable concentration in commercial soil	Bioavailable concentration in Martian regolith simulant
Mg	ppm	430.7	4626.2
Ca	ppm	5931.2	4650.4
K	ppm	677.3	3.3
P	ppm	192.5	<0.5
N	% (m/m)	0.442	0.019
Organic carbon	% (m/m)	41.4	0.08

Table 2. Percentage differences (%) in the average values of the measured growth parameters in the negative controls of the watermelon plants grown in commercial soil and a Martian regolith simulant. Positive values indicate higher values in the soil. Letters in brackets (a) and (b) indicate statistically significant differences; values which share the same letter are not significantly different. The significance threshold was set at $\alpha = 0.05$.

Parameter	Soil		Regolith		Relative difference (%) (Soil vs. Regolith)
	Mean	SD	Mean	SD	
Height (cm)	17.38 (a)	2.55	11.7 (b)	0.98	32.67%
Root length (cm)	5.02 (a)	2.14	2.34 (b)	1.18	53.45%
No of leaves	2.91 (a)	0.83	1.5 (b)	0.53	48.45%
Total leaf area (cm ²)	18.84 (a)	10.82	1.43 (b)	0.71	92.43%
Fresh weight (g)	1.79 (a)	0.53	0.575 (b)	0.07	67.87%
ChlA (mg/L)	0.99 (a)	0.24	0.94 (a)	0.15	5.39%
ChlB (mg/L)	4.33 (a)	0.95	4.08 (a)	0.57	5.75%
Chl total (mg/L)	14.05 (a)	3.03	13.1 (a)	1.52	6.76%

RESULTS

The chemical composition of the regolith simulant differed markedly from that of the soil substrate (Table 1). While magnesium was present at higher concentrations in the simulant compared to the soil, calcium was slightly lower. Potassium, phosphorus, nitrogen, and organic carbon were drastically reduced in the simulant, with phosphorus and organic carbon almost absent. Overall, the regolith simulant was severely depleted in essential nutrients and organic matter compared to the soil, reflecting a harsh, nutrient-poor environment likely to limit plant growth without supplementation.

After four weeks of growth in the Martian regolith simulant and soil substrate, the watermelon plants were more developed in the soil compared to those grown in the regolith (Fig. 1). The first true leaves emerged 11 days after sowing in the soil, whereas leaf emergence was delayed until after 18 days in the regolith substrate. Seed germination, assessed by the number of emerged plants relative to the number of seeds sown, was affected by treatment (Supplementary Table 1). In the regolith, 78.6% of the seeds germinated in the negative control, while 100% germinated with the Bo+Do+F4 treatment and 92.9% with the Do+F4 treatment. In the soil, 78.6% of the seeds germinated in the negative control compared with 85.7% with the Bo+Do treatment. The F4 treatment alone and the Bo+Do+F4 combination neither enhanced nor suppressed germination in the soil.

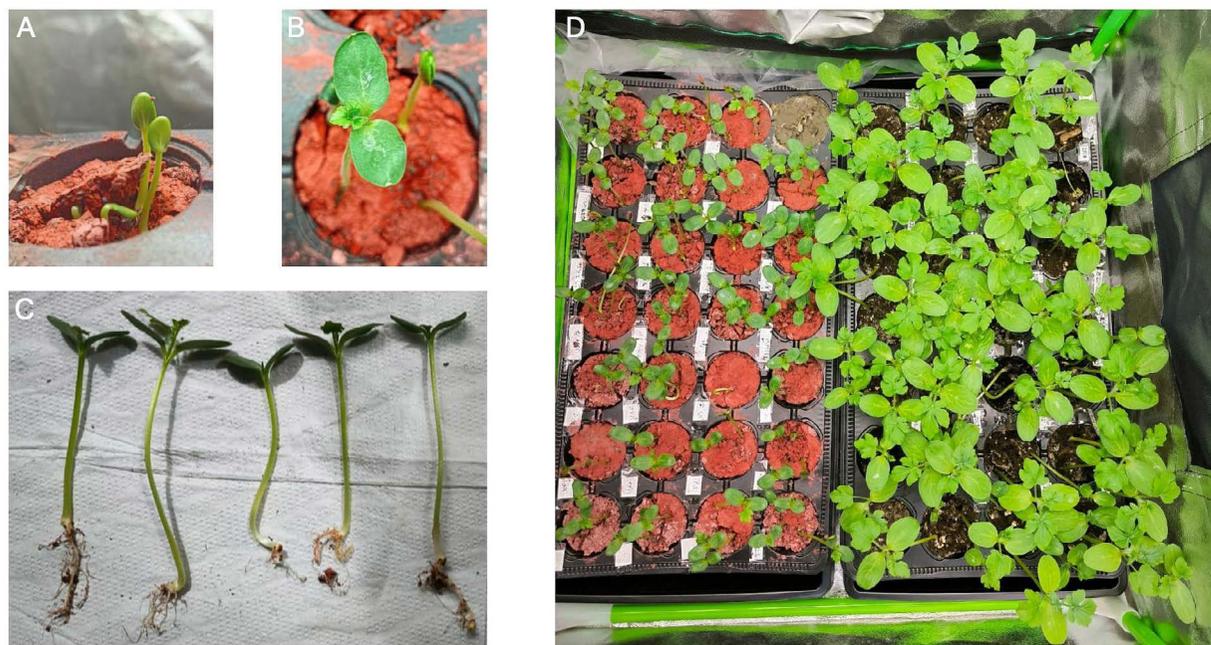


Fig. 1. Representative images of the watermelon plants grown in a Martian regolith simulant and commercial soil. **A.** Emergence of the watermelon seedlings through the regolith surface, 6 days after sowing; **B.** Appearance of the first true leaves in the plants grown in regolith, 18 days after sowing; **C.** Watermelon plants after 4 weeks of growth in the Martian regolith; **D.** Comparison of the watermelon plants grown in the regolith and soil, 4 weeks after sowing.

Permutational multivariate analysis of variance indicated statistically significant differences in the measured parameters due to the substrate type, treatment, and their interaction ($p < 0.05$) (Supplementary Table 2). The effect of the substrate was the most pronounced, particularly influencing the plant height, root length, number of leaves, total leaf area, and fresh weight (Fig. 2). Post-hoc pairwise tests revealed no statistically significant differences in the chlorophyll content between the soil- and regolith-grown plants, indicating that chlorophyll biosynthesis may not be strongly impacted by substrate composition (Supplementary Table 3). Based on the measured growth parameters in the negative controls after four weeks, the most noticeable and statistically significant differences were observed in the total leaf area (92.43%) and fresh weight (67.87%) (Table 2).

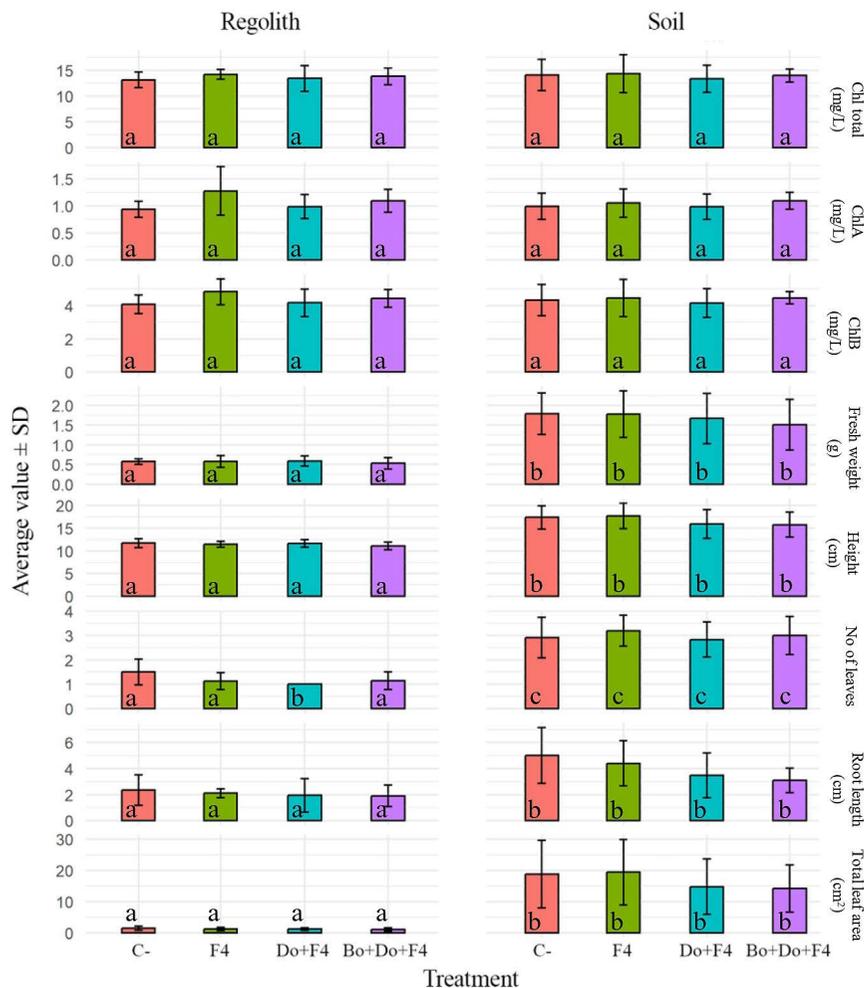
When analysing the effect of the treatments within the regolith substrate, the only statistically significant difference compared to the negative control was observed in the number of leaves (Fig. 2; Supplementary Table 4). Specifically, the plants treated with Do+F4 had fewer leaves than those in the negative control.

DISCUSSION

The significance of this investigation lies in demonstrating the ability of watermelon plants to grow under challenging conditions, specifically in a nutrient-poor substrate designed to mimic the composition and limitations of Martian soil. The substrate contained the essential macronutrients, nitrogen, phosphorus, and potassium, at extremely low concentrations, likely originating from impurities in the substances used for its preparation. In contrast, the bioavailable levels of secondary nutrients, magnesium and calcium, were high, with magnesium much higher than typically found in soil and calcium similar to natural soil levels. The transformation of these compounds from oxides into plant-accessible forms may have been facilitated by naturally occurring bacteria in the non-sterilised substrate (FACKRELL *et al.* 2024).

The delay in the development of the first true leaves in the regolith simulant suggests a prolonged dependence on seed nutrient reserves, highlighting

Fig. 2. Average values of growth and physiological parameters (y-axis) in the watermelon plants originating from the seeds treated with plant growth-promoting bacteria (x-axis), cultivated in two different substrates: a Martian regolith simulant and commercial soil. Letters placed inside or above the bars indicate statistically significant differences among the treatments and substrates for each measured parameter.



the slow initial growth under extreme nutrient-poor conditions. The delayed emergence of true leaves suggests that the watermelon seedlings relied heavily on the limited resources stored in the seed endosperm during early development. Continued growth beyond this stage implies that external sources, albeit minimal, supported further plant development despite the unfavourable conditions. It is also important to note that the tap water used for watering is likely to have provided small amounts of nutrients, as ions such as magnesium, calcium, potassium, and bicarbonates are present in the public water supplies used in our study (PETROVIĆ *et al.* 2012).

Interestingly, the chlorophyll content was similar between the plants grown in soil and those grown in the regolith simulant. Chlorophyll is a crucial pigment in the photosynthetic apparatus, responsible for light absorption and photochemical reactions (LI *et al.* 2024). Nitrogen, particularly in the form of nitrate (NO_3), is one of the most important nutrients for plant growth and development, and it plays a key role in chlorophyll synthesis (GOU *et al.* 2020). Early chlorophyll formation can be attributed to nitrogen remobilised from seed storage proteins. However, given the four-week growth period, seed-derived nitrogen alone is unlikely to have fully sustained chlorophyll maintenance, suggesting that trace nitrogen inputs and efficient internal nitrogen recycling contributed to prolonged plant viability under nitrogen-limited conditions. It has been shown that chlorophyll synthesis can be enhanced under nitrate stress in the presence of silicon (GOU *et al.* 2020), which could

have occurred due to the silicon abundance in Martian regolith simulants, primarily as SiO₂. EICHLER *et al.* (2021) also reported similar chlorophyll levels in plants grown in the JSC-Mars-1A and MMS-1 Martian regolith simulants compared to those grown in soil. However, in another study, the addition of compost to a Mars regolith simulant significantly increased the chlorophyll a and b content compared to the plants grown in the pure simulant substrate (DURI *et al.* 2020).

The application of microorganisms to solubilise essential elements in extraterrestrial simulants is a promising strategy for improving substrate fertility. The strains we used for seed treatment are known for their tolerance to heavy metals and exhibit various plant growth-promoting traits (RAKIĆ *et al.* 2021), among which indole-3-acetic acid (IAA) and siderophore production may be of particular importance for this study. Bacterial siderophores, which chelate and mobilise iron from mineral-rich phases, may facilitate iron redistribution within the regolith, thereby improving the accessibility of this essential micronutrient to plants (FACKRELL *et al.* 2024), while the production of IAA is known to enhance seed germination and early seedling growth in *Cucurbitaceae* (BLINKOV *et al.* 2014). The most notable effect of the applied PGPB was observed during the germination phase in the regolith simulant, where various combinations of strains promoted successful germination in a significantly higher number of seeds compared to the untreated control. These findings suggest that certain bacterial treatments may enhance germination even in substrates of suboptimal composition. Utilising PGPB consortia rather than individual strains appears to be a more effective approach due to their enhanced adaptability, robustness, and multifunctionality (WOO & PEPE 2018; KAUSHAL *et al.* 2023). To ensure the success of a microbial inoculant, its viability should be monitored, as effective colonisation of external or internal plant tissues is required; however, even then, its long-term persistence is not guaranteed (ROMANO *et al.* 2020). Therefore, various approaches should be employed to track bioinoculants over time, including culture-dependent, microscopic, and molecular methods (ROMANO *et al.* 2020). Cultural methods, such as plate counting on selective or non-selective media, provide direct evidence of cell viability through colony formation. To distinguish the viable cells of the target bacterial species from the non-viable cells, methods combining species specificity with viability assessment can be applied. Fluorescence *in situ* hybridisation using fluorescently labelled oligonucleotide probes enables the direct visualization of specific bacterial taxa, and when combined with LIVE/DEAD staining, it allows the simultaneous evaluation of both cell integrity and species identity (SAVICHTCHEVA *et al.* 2005). Alternatively, propidium monoazide treatment coupled with qPCR or dPCR selectively amplifies DNA from intact (viable) cells, while excluding signals from membrane-compromised (non-viable) cells (TAKAHASHI *et al.* 2018; OKADA *et al.* 2022).

The choice of the most appropriate PGPB depends on various factors such as plant species, its growth method, and the location of the potential Martian colony site (HANDY *et al.* 2021), as well as the specific characteristics of the individual PGPB strains. The continuous search for more effective PGPB candidates remains essential, but even the best strains may express limited efficacy in substrates lacking the organic matter and essential nutrients for plant growth. In the study carried out by DURI *et al.* (2025) where a Martian regolith simulant was mixed with compost, the application of PGPB enhanced plant growth across all the measured parameters and resulted in increased nutrient content in the plant tissue. Strategies which incorporate varying amounts of composted organic waste to enrich the regolith and transform it into a more life-sustaining substrate may represent a promising approach. Importing organic material from Earth to Mars is not feasible, so the addition

of *in situ* recycled organic matter to enrich regolith (improving fertility, structure, and water-holding capacity) represents a sustainable solution. Organic waste generated by crew members can be processed as compost or fertiliser to support plant growth. Future studies should evaluate the effectiveness of these treatments under Martian conditions.

CONCLUSION

The growth of watermelon plants in a substrate containing oxides which resemble a Martian regolith simulant is achievable to some extent. The application of PGPB consortia during the seed stage appears to be a promising strategy for enhancing seed germination under such conditions. However, the low-nutrient environment of the regolith is likely to pose a specific stress limiting plant development compared to growth in nutrient-rich soil. The incorporation of organic matter into the regolith substrate could potentially mitigate this effect and promote better growth. Interestingly, certain physiological parameters, such as the chlorophyll content, remained stable regardless of the substrate, suggesting that photosynthetic function is preserved even under stress. This study represents a preliminary step in the broader field of space agriculture, where a comprehensive understanding of abiotic stressors is essential to simulate realistic conditions. Continued research is needed to develop sustainable plant cultivation systems capable of supporting bioregenerative life support systems and ultimately aiding the human colonisation of space.

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REFERENCES

- BEECH M & COMTE M. 2021. Life on Mars: Past, Present, and Future. In: BEECH M, SECKBACH J & GORDON R (eds.), *Terraforming Mars*, pp. 135–160, Scrivener Publishing LLC, Beverly. <https://doi.org/10.1002/9781119761990.ch9>
- BISHOP JL, MURCHIE SL, PIETERS CM & ZENT AP. 2002. A model for formation of dust, soil, and rock coatings on Mars: Physical and chemical processes on the Martian surface. *Journal of Geophysical Research: Planets* **107**: 5097. <https://doi.org/10.1029/2001JE001581>
- BLINKOV EA, TSAVKELOVA EA & SELITSKAYA OV. 2014. Auxin production by the *Klebsiella planticola* strain TSKhA-91 and its effect on development of cucumber (*Cucumis sativus* L.) seeds. *Microbiology* **83**: 531–538. <https://doi.org/10.1134/S0026261714050063>
- DURI LG, CAPORALE AG, ROUPHAEL Y, VINGIANI S, PALLADINO M, DE PASCALE S & ADAMO P. 2022. The potential for lunar and martian regolith simulants to sustain plant growth: a multidisciplinary overview. *Frontiers in Astronomy and Space Sciences* **8**: 278–293. <https://doi.org/10.3389/fspas.2021.747821>
- DURI LG, EL-NAKHEL C, CAPORALE AG, CIRIELLO M, GRAZIANI G, PANNICO A, PALLADINO M, RITIENI A, DE PASCALE S, VINGIANI S & ROUPHAEL Y. 2020. Mars regolith simulant ameliorated by compost as *in situ* cultivation substrate improves lettuce growth and nutritional aspects. *Plants* **9**: 628. [10.3390/plants9050628](https://doi.org/10.3390/plants9050628)

- DURI LG, ROMANO I, ADAMO P, ROUPHAEL Y, PANNICO A, VENTORINO V, PEPE O, DE PASCALE S & CAPORALE AG. 2025. From earth to space: how bacterial consortia and green compost improve lettuce growth on lunar and martian simulants. *Biology and Fertility of Soils* **61**: 1145–1164. <https://doi.org/10.1007/s00374-025-01923-3>
- FACKRELL LE, HUMPHREY S, LOUREIRO R, PALMER AG & LONG-FOX J. 2024. Overview and recommendations for research on plants and microbes in regolith-based agriculture. *npj Sustainable Agriculture* **2**: 15. <https://doi.org/10.1038/s44264-024-00013-5>
- FERL R, WHEELER R, LEVINE HG & PAUL AL. 2002. Plants in space. *Current Opinion in Plant Biology* **5**: 258–263. [https://doi.org/10.1016/S1369-5266\(02\)00254-6](https://doi.org/10.1016/S1369-5266(02)00254-6)
- EICHLER A, HADLAND N, PICKETT D, MASAITIS D, HANDY D, PEREZ A, BATCHELDOR D, WHEELER B & PALMER A. 2021. Challenging the agricultural viability of martian regolith simulants. *Icarus* **354**: 114022. <https://doi.org/10.1016/j.icarus.2020.114022>
- GILLMANN C, LOGNONNÉ P, CHASSEFIÈRE E & MOREIRA M. 2009. The present-day atmosphere of Mars: Where does it come from? *Earth and Planetary Science Letters* **277**: 384–393. <https://doi.org/10.1016/j.epsl.2008.10.033>
- GLICK BR. 2012. Plant growth-promoting bacteria: mechanisms and applications. *Scientifica* **2012**: 963401. <https://doi.org/10.6064/2012/963401>
- GOU T, YANG L, HU W, CHEN X, ZHU Y, GUO J & GONG H. 2020. Silicon improves the growth of cucumber under excess nitrate stress by enhancing nitrogen assimilation and chlorophyll synthesis. *Plant Physiology and Biochemistry* **152**: 53–61. <https://doi.org/10.1016/j.plaphy.2020.04.031>
- HANDY D, HUMMERICK ME, DIXIT AR, RUBY AM, MASSA G & PALMER A. 2021. Identification of plant growth promoting bacteria within space crop production systems. *Frontiers in Astronomy and Space Sciences* **8**: 201–210. <https://doi.org/10.3389/fspas.2021.735834>
- HARRIS F, DOBBS J, ATKINS D, IPPOLITO JA & STEWART JE. 2021. Soil fertility interactions with *Sinorhizobium*-legume symbiosis in a simulated Martian regolith; effects on nitrogen content and plant health. *PLoS One* **16**: e0257053. <https://doi.org/10.1371/journal.pone.0257053>
- KANE M & SHASTEEN KC. 2024. Importance and challenges of integrating BLSS into ECLSS. *Acta Astronautica* **220**: 185–196. <https://doi.org/10.1016/j.actaastro.2024.04.016>
- KAUSHAL M, DEVI S, KUMAWAT KC & KUMAR A. 2023. Microbial consortium: a boon for a sustainable agriculture. In: PARRAY JA (ed.), *Climate Change and Microbiome Dynamics: Carbon Cycle Feedbacks*, pp. 15–31, Springer International Publishing, Cham. https://doi.org/10.1007/978-3-031-21079-2_2
- LI X, ZHANG W, NIU D & LIU X. 2024. Effects of abiotic stress on chlorophyll metabolism. *Plant Science* **342**: 112030. <https://doi.org/10.1016/j.plantsci.2024.112030>
- LONG-FOX JM & BRITT DT. 2023. Characterization of planetary regolith simulants for the research and development of space resource technologies. *Frontiers in Space Technologies* **4**: 7–22. <https://doi.org/10.3389/frspt.2023.1255535>
- MAGGI F & PALLUD C. 2010. Martian base agriculture: The effect of low gravity on water flow, nutrient cycles, and microbial biomass dynamics. *Advances in Space Research* **46**: 1257–1265. <https://doi.org/10.1016/j.asr.2010.07.012>
- MAITY T & SAXENA A. 2024. Challenges and innovations in food and water availability for a sustainable Mars colonization. *Life Sciences in Space Research* **42**: 27–36. <https://doi.org/10.1016/j.lssr.2024.03.008>
- MAOTO MM, BESWA D & JIDEANI AI. 2019. Watermelon as a potential fruit snack. *International Journal of Food Properties* **22**: 355–370. <https://doi.org/10.1080/10942912.2019.1584212>
- MARTINEZ ARBIZU P. 2020. pairwiseAdonis: Pairwise multilevel comparison using adonis. R package version 0.4. <https://github.com/pmartinezarbizu/pairwiseAdonis>
- McSWEEN Jr HY & KEIL K. 2000. Mixing relationships in the Martian regolith and the composition of globally homogeneous dust. *Geochimica et Cosmochimica Acta* **64**: 2155–2166. [https://doi.org/10.1016/S0016-7037\(99\)00401-9](https://doi.org/10.1016/S0016-7037(99)00401-9)
- OKADA A, TSUCHIDA M, RAHMAN MM & INOSHIMA Y. 2022. Two-round treatment with propidium monoazide completely inhibits the detection of dead *Campylobacter* spp. cells by quantitative PCR. *Frontiers in Microbiology* **13**: 801961. <https://doi.org/10.3389/fmicb.2022.801961>

- OKSANEN J, BLANCHET FG, FRIENDLY M, KINDT R, LEGENDRE P, MCGLINN D, MINCHIN PR, O'HARA RB, SIMPSON GL, SOLYMOS P, STEVENS MHH, SZOEC S & WAGNER H. 2022. *vegan: Community Ecology Package*. R package version 2.6-4. <https://CRAN.R-project.org/package=vegan>
- PETERS GH, ABBEY W, BEARMAN GH, MUNGAS GS, SMITH JA, ANDERSON RC, DOUGLAS S & BEEGLE LW. 2008. Mojave Mars simulant—Characterization of a new geologic Mars analog. *Icarus* **197**: 470–479. <https://doi.org/10.1016/j.icarus.2008.05.004>
- PEŠIĆ M, RADOVIĆ S, RAKIĆ T, DŽELETOVIĆ Ž, STANKOVIĆ S & LOZO J. 2024. Insights into the response of *Miscanthus × giganteus* to rhizobacteria: Enhancement of metal tolerance and root development under heavy metal stress. *Archives of Biological Sciences* **76**: 205–221. <https://doi.org/10.2298/ABS240301014P>
- PETROVIĆ TM, ZLOKOLICA-MANDIĆ M, VELJKOVIĆ N, PAPIĆ PJ, POZNANOVIĆ MM, STOJKOVIĆ JS & MAGAZINOVIĆ SM. 2012. Macro and microelements in bottled and tap waters of Serbia. *Hemijska Industrija* **66**: 107–122. <https://doi.org/10.2298/HEMIND110729062P>
- R CORE TEAM. 2024. *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing, Vienna, Austria. <https://www.R-project.org/>
- RAKIĆ T, PEŠIĆ M, KOSTIĆ N, ANDREJIĆ G, FIRA D, DŽELETOVIĆ Ž, STANKOVIĆ S & LOZO J. 2021. *Rhizobacteria* associated with *Miscanthus x giganteus* improve metal accumulation and plant growth in the flotation tailings. *Plant and Soil* **462**: 349–363. <https://doi.org/10.1007/s11104-021-04865-5>
- RAMÍREZ DA, KREUZE J, AMOROS W, VALDIVIA-SILVA JE, RANCK J, GARCIA S, SALAS E & YACTAYO W. 2019. Extreme salinity as a challenge to grow potatoes under Mars-like soil conditions: targeting promising genotypes. *International Journal of Astrobiology* **18**: 18–24. <https://doi.org/10.1017/S1473550417000453>
- RITCHIE RJ. 2008. Universal chlorophyll equations for estimating chlorophylls a, b, c, and d and total chlorophylls in natural assemblages of photosynthetic organisms using acetone, methanol, or ethanol solvents. *Photosynthetica* **46**: 115–126. <https://doi.org/10.1007/s11099-008-0019-7>
- ROMANO I, VENTORINO V & PEPE O. 2020. Effectiveness of plant beneficial microbes: overview of the methodological approaches for the assessment of root colonization and persistence. *Frontiers in Plant Science* **11**: 232–247. <https://doi.org/10.3389/fpls.2020.00006>
- SÁEZ-PLAZA P, NAVAS MJ, WYBRANIEC S, MICHAŁOWSKI T & ASUERO AG. 2013. An overview of the Kjeldahl method of nitrogen determination. Part II. Sample preparation, working scale, instrumental finish, and quality control. *Critical Reviews in Analytical Chemistry* **43**: 224–272. <https://doi.org/10.1080/10408347.2012.751787>
- SAVICHTCHEVA O, OKAYAMA N, ITO T & OKABE S. 2005. Application of a direct fluorescence-based live/dead staining combined with fluorescence in situ hybridization for assessment of survival rate of *Bacteroides* spp. in drinking water. *Biotechnology and Bioengineering* **92**: 356–363. <https://doi.org/10.1002/bit.20608>
- SCHNEIDER CA, RASBAND WS & ELICEIRI KW. 2012. NIH Image to ImageJ: 25 years of image analysis. *Nature Methods* **9**: 671–675. <https://doi.org/10.1038/nmeth.2089>
- SHAMRIKOVA EV, KONDRATENOK BM, TUMANOVA EA, VANCHIKOVA EV, LAPTEVA EM, ZONOVA TV, LU-LYAN-MIN EI, DAVYDOVA AP, LIBOHOVA Z & SUVANNANG N. 2022. Transferability between soil organic matter measurement methods for database harmonization. *Geoderma* **412**: 115547. <https://doi.org/10.1016/j.geoderma.2021.115547>
- SINGH M, SINGH P, SINGH S, SAINI RK & ANGADI SV. 2021. A global meta-analysis of yield and water productivity responses of vegetables to deficit irrigation. *Scientific Reports* **11**: 22095. [10.1038/s41598-021-01433-w](https://doi.org/10.1038/s41598-021-01433-w)
- TAKAHASHI H, KASUGA R, MIYA S, MIYAMURA N, KUDA T & KIMURA B. 2018. Efficacy of propidium monoazide on quantitative real-time PCR-based enumeration of *Staphylococcus aureus* live cells treated with various sanitizers. *Journal of Food Protection* **81**: 1815–1820. [10.4315/0362-028X.JFP-18-059](https://doi.org/10.4315/0362-028X.JFP-18-059)
- WEHNER TC. 2008. Watermelon. In: PROHENS J & NUEZ F (eds.), *Vegetables I: Asteraceae, Brassicaceae, Chenopodiaceae, and Cucurbitaceae*, pp. 381–418, Springer, New York. https://doi.org/10.1007/978-0-387-30443-4_12

- WICKHAM H. 2016. *ggplot2: Elegant Graphics for Data Analysis*. Springer-Verlag, New York. <https://ggplot2.tidyverse.org>
- WICKHAM H, FRANÇOIS R, HENRY L & MÜLLER K. 2023. *dplyr: A Grammar of Data Manipulation*. R package version 1.1.4. <https://CRAN.R-project.org/package=dplyr>
- WOO SL & PEPE O. 2018. Microbial consortia: promising probiotics as plant biostimulants for sustainable agriculture. *Frontiers in Plant Science* **9**: 435–440. <https://doi.org/10.3389/fpls.2018.01801>



REZIME

Gajenje lubenice (*Citrullus lanatus*) u simulantu Marsovog regolita nakon tretmana semena bakterijama koje pospešuju rast biljaka

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Kolonizacija svemira od strane ljudi više nije ograničena na domen naučne fantastike. Među različitim nebeskim telima, Mars se izdvojio kao primarna meta za dugoročno ljudsko naseljavanje u budućnosti. Jedan od glavnih izazova u takvim misijama predstavlja obezbeđivanje održivog izvora hrane. Transport hrane na tako velike udaljenosti je skup i nepraktičan, što podstiče sve veće interesovanje za razvoj poljoprivrede u svemiru i korišćenje *in situ* resursa za gajenje biljaka, kao što je regolit na površini Marsa. U ovoj studiji istraživali smo mogućnost za uzgajanje lubenice (*Citrullus lanatus*) u simuliranim marsovskim uslovima koristeći supstrat koji oponaša sastav marsovskog regolita. Da bi se ublažio nedostatak hranljivih materija, seme je tretirano bakterijama koje podstiču rast biljaka (PGPB) izolovanim iz rizosfere *Miscanthus × giganteus*, biljke gajene na zemljištu kontaminiranom teškim metalima. Statistički značajne razlike u parametrima rasta – uključujući visinu, dužinu korena, svežu masu i površinu lista – zabeležene su između biljaka gajenih u zemljištu i regolitu, dok u sadržaju hlorofila nisu zabeležene značajne varijacije, što ukazuje na očuvanu fotosintetičku funkciju uprkos abiotičkom stresu. Pored toga, semena inokulisana bakterijskim konzorcijumom imala su poboljšanu klijavost u poređenju sa netretiranom kontrolom. Ovo istraživanje predstavlja prvi pokušaj gajenja lubenice u simulantu marsovskog regolita i ukazuje na potencijal upotrebe PGPB kao obećavajuće strategije za unapređenje performansi biljaka u ekstremnim uslovima. Dalja istraživanja su neophodna u cilju optimizacije sastava konzorcijuma mikroorganizama i regolita za buduće primene u svemirskoj poljoprivredi.

Ključne reči: BLSS, ISRU, konzorcijum mikroorganizama, svemirska poljoprivreda, kolonizacija svemira