

Growth promoting bacterium *Mokoshia rubra* isolated from Antarctic soil stimulates growth and physiological parameters of *Arabidopsis thaliana* cultivated in lunar regolith analog

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Abstract

With the planning, preparation, and execution of future long-term lunar missions, there is a growing need to develop methods that enable sustainable plant cultivation in lunar regolith, as future lunar greenhouses will require reliable and safe cultivation technologies. Over the past two decades, research has focused on the comprehensive evaluation of lunar regolith as a potential plant growth substrate and on strategies to improve its properties. These studies have investigated the addition of nutrients and organic amendments, as well as the application of autotrophic and heterotrophic microorganisms, particularly plant growth-promoting bacteria. The present study evaluated the effects of three amendments to a lunar regolith analogue: (1) Hoagland nutrient solution, (2) biochar, and (3) the plant growth-promoting bacterium *Mokoshia rubra*. Their effects on photosynthetic performance, growth, and physiological parameters of *Arabidopsis thaliana* were investigated. Hoagland nutrient solution and *M. rubra* inoculation positively affected the majority of chlorophyll fluorescence parameters related to PSII function and efficiency.

Key words: James Ross Island, space agriculture, plants for space, GPB, *Mokoshia rubra*

Abbreviations: ChlF – chlorophyll fluorescence, DM – dry mass, GPB – growth promoting bacteria, LA – leaf area

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Introduction

Lunar regolith is a particulate and porous material that covers the lunar surface (Shkuratov and Bondarenko 2001, Qin *et al.* 2025). It consists of several constituents, mainly plagioclase, olivine, augit, pigeonit, and glass (Taylor *et al.* 2019). The major constituents of lunar regolith include SiO₂, Al₂O₃, FeO, MgO, and CaO. The share of them, however, differs in particular regoliths between collection sites (Lunar highlands *versus* the mare sites – Zhou *et al.* 2022). Lunar regoliths are considered as potential substrates for plant cultivation in future infrastructure of lunar stations because they represent a solid substrate and a source of inorganic nutrients (Wamelink *et al.* 2014). Lunar regolith has recently attracted considerable attention as a potential building material for civil engineering in future lunar missions and constructions, the energy and mineral sources for lunar industry and medicine. Furthermore, lunar regolith is considered raw materials for lunar agriculture (for review *see* Zhang *et al.* 2021).

Lunar regolith, however, exhibits very low cation-exchange capacity and lack clay minerals and organic matter, limiting nutrient retention and buffering capacity (Antonangelo *et al.* 2024).

Moreover, growth of many plant species in lunar regolith and/or lunar regolith simulants *per se* is negatively affected by mainly nutrient limitation, heavy metal ions resulting in growth rate reduction (Paul *et al.* 2022), and negative adjustment of several physiological processes including photosynthesis and modification of the expression of genes involved in photosynthesis (Handy *et al.* 2026). Due to the limited availability of authentic lunar regolith, either commercial lunar regolith simulants or natural analogs such as *e.g.* regolith originating from the Earth volcanic rocks are used. Recent research focused on experimental plant cultivation moved rather to lunar regolith analogues/simulants (Ar-

slan *et al.* 2010, Anbazhagan *et al.* 2021, Ding *et al.* 2024) since only limited amount of authentic lunar regolith is available for the on-Earth experiments. Within last few years, various types of substrates simulating lunar regolith have been developed for different research purposes. Recently, more than 150 different analogues are reported in literature (*see* Table 1s, Supplementary Materials).

Experiments that have been made with *Arabidopsis thaliana* clearly demonstrated that the species may germinate and grow in both lunar regolith and/or its simulants potential feasibility of lunar agriculture (Wamelink *et al.* 2014, 2019). However, *A. thaliana* plants grown in lunar regolith show a great variety of irregularities. The data presented by Paul *et al.* (2022) for the lunar regolith showed slowed plant growth and development with signs of severe stress morphologies. The study found that *A. thaliana* grown in lunar regolith differentially expressed genes indicating ionic stresses. Moreover, the plants exhibit a higher degree of genome oxidation and decreased biomass relative to conventional Earth soil cultivation (Barcenilla *et al.* 2024). The same study reports a decline in telomerase activity and telomeres shortening in plants grown in lunar regolith simulant.

Recent studies suggest that plant growth promoting bacteria (GPB), such as *Bacillus*, *Pseudomonas*, and *Azotobacter*, can turn nutrient-poor lunar regolith into a viable growth medium by increasing nutrient bioavailability (*e.g.*, phosphorus solubilization) and reducing environmental stress on plants. Duri *et al.* (2025) investigated the role of selected consortium of GPB formed by *Azotobacter chroococcum* 76A, *Priestia megaterium* EL5, *Methylobacterium populi* VP2, and *Kosakonia pseudosacchari* TL13 in enhancing plant growth on Martian and Lunar regolith simulants. The study concluded that the microbial consortium along

with green compost is a promising strategy to improve regolith simulants fertility and thus, plant development, passing nutrient-deficient conditions. Similarly, Mei et al. (2026) reported that consortium of *Bacillus*, *Glutamicibacter*, *Acetobacter*, *Enterococcus*, and *Microbacterium* increased the contents of available nutrients (N, P, and K) and organic matter in simulated lunar soil, thereby enhancing lettuce growth and biomass accumulation. The study reported that the treatment with the above consortium led to an increase chlorophyll contents, and alleviated oxidative stress by improving the antioxidant system.

This study addresses the use of Antarctic regolith as a tool for lunar simulant, as well as provide a more novel insights on the In Situ Resource Utilization (ISRU) in the Antarctic continent. Previous studies al-

ready explored the utilization of Antarctic regolith as lunar analog (Haq et al. 2026a), however, for practical applications there is the necessity to further evaluate how soil amendments such as biochar or local resources such as Antactic bacteria strains may constitute a tool to improve the quality of the substrate, allowing an improved cultivation of crops.

In this study, we investigated the the effectt of Antarctic bacterium (*Mokoshia rubra*) on growth and photosynthetic parameters of *Arabidopsis thaliana*. We hypothesized that, similarly to the earlier study made on *N. tabacum* grown in soil (Haq et al. 2026b), addition of the bacterium would improve growth and photosynthetic characteristics of the experimental plants. Apart of *M. rubra* addition, we also tested the effect of a biochar addition.

Material and Methods

Antarctic regolith characteristics

Antarctic regolith was collected from the foothill of Northern slopes of Berry Hill mesa (57° 50′ 19″ W, 63° 48′ 54″ S) at the James Ross Island (Antarctica). The collected regolith was sieved on site to gain 1 mm fraction and dried at room temperature at the Mendel station (Czech Antarctic Research Programme facility). Chemical composition of the regolith is given in Table 1. Since the regolith is of

volcanic origin, it contains heavy metals Cd, Cr, Cu, Ni, Pb, and Zn (Coufalík et al. 2024).

After the transfer to the Masaryk University (Brno, CZ) laboratories, the regolith was used as lunar regolith analog for experimental plant cultivation and evaluation of different treatment effects on plant growth, vitality and physiological performance.

Na ₂ O	MgO	Al ₂ O ₃	SiO ₂	P ₂ O ₅	SO ₃	Cl
2.69	7.46	18.66	55.30	0.33	0.00	0.00
K ₂ O	CaO	TiO ₂	Cr ₂ O ₃	MnO	FeO (Fe ₂ O ₃)*	total
1.40	4.09	1.56	0.00	0.00	8.51	100.00

Table 1. Relative contents (%) of selected elements in the Antarctic regolith collected at James Ross Island used in this study (adopted from Coufalík et al. 2024).

Cultivation of growth promoting bacterium

Mokoshia rubra (strain CCM 8692^T) was provided by the Czech Collection of Microorganisms (Brno, Czech Republic^[1]). The strain was originally isolated from the substrates on northern, deglaciated part of James Ross Island, Antarctica and described as *species nova* by Holochová *et al.* (2020). For culturing, the CCM 8692^T strain was inoculated to R2A broth (Oxoid) and incubated at 20°C with continuous

shaking (150 rpm) to avoid the formation of clumps. The cultivation lasted 48 hours to reach the logarithmic growth phase. Then, the bacterial suspension was standardized to an OD600 of 0.8–1.0, corresponding to approximately 10⁸ CFU ml⁻¹ (colony-forming unit), using a spectrophotometer. Bacterial suspension was applied to the regolith surface.

Plant cultivation

Seeds of *Arabidopsis thaliana* (Col-0, wild type) were obtained from the Laboratory of Photosynthetic Processes, Masaryk University, Brno, Czech Republic. After sterilization in 70% alcohol for 2 min., seeds were sown for one week in a previously autoclaved soil mixture composed of 12 parts of peat, 2 parts of sand, and 1 part of perlite, for one week. The soil mixture was autoclaved before utilisation. Germination was conducted in a greenhouse with

the following parameters: temperature of 17/28°C min/max, average of 22°C; relative humidity was 60/65% day/night; light intensity of 150 ± 10 µmol(photon) m⁻² s⁻¹ with a 14/10 h day/night photoperiod. After 2 weeks early germinating individuals of about 3 mm in size were available; then (day 0), the plants were transplanted into 4 regolith-based substrates differing in their characteristics (additions/watering) – see Table 2.

Group No.	Abbreviation	Biochar addition	Bacterial strain <i>Mokoshia rubra</i>	Watering
1	W	no	no	Demineralized water
2	H	no	no	Hoagland solution
3	W+B	yes	no	Demineralized water
4	H+B	yes	no	Hoagland solution
5	W+M	no	yes	Demineralized water
6	H+M	no	yes	Hoagland solution
7	W+B+M	yes	yes	Demineralized water
8	H+B+M	yes	yes	Hoagland solution

Table 2. Overview of the treatments used in this study focused on *Arabidopsis thaliana* growth in Lunar regolith simulant, B – biochar, M – *Mokoshia rubra*, W – water, H – Hoagland solution.

Individual plants were cultivated in 15 ml-in-volume holes of a tissue culture testplates (TRP 92012, Switzerland, see Fig. 1) filled in by the 1-8 substrates specified in Table 2. For *M. rubra* inoculation (the treatments 5-8), the above-specified

suspension was introduced into autoclaved (wet) regolith by adding 3 ml of the inoculum per hole. To avoid water supply insufficiency, the plants were watered regularly (typically in 2-3 days interval) by 6 ml of either demineralized water or Hoag-

land growth solution (for the composition, *see* Table 2s, Suppl. Materials). During the cultivation time (day 0 to day 39) cultivation plates were covered by transparent plastic cover sized $12.6 \times 8.2 \times 11.2$ cm ($l \times w \times h$) to prevent rapid evaporation of water/Hoagland solution (for details *see* Krejčí et al. 2026). Such cultivation system was placed into a controlled growth

chamber (VAHA, Kladno, Czech Republic), where the plants were cultivated at 21°C, and incident photosynthetically active radiation (PAR) of $120 \mu\text{mol m}^{-2} \text{s}^{-1}$ for 40 d. During the cultivation time, photographs were taken regularly (typically in 5 d interval) to document differences in growth rate and leaf area development for individual treatments.

Primary photosynthesis

Treatment-, and time of cultivation-dependent changes in primary photosynthetic processes were assessed by several chlorophyll fluorescence (ChlF) parameters. For the approach of chlorophyll fluorescence imaging and a FluorCam instrument (Open FluorCam FC 800, Photon Systems Instruments, Czech Republic) were used. In 3-5 days interval, slow Kautsky kinetics (KK), supplemented with quenching analysis, was measured by the technique described by Barták et al. (2023). Prior to measurements, plants were dark-adapted for 15 min. to allow PSII centres to reach fully open state, *i.e.* maximize capacity of energy flow through PSII to the system of electron carriers forming linear electron transport chain. The following chlorophyll fluorescence parameters were evaluated for each single plant:

- (1) maximum quantum yield

$$F_V / F_M = F_M - F_0 / F_M$$

(Genty et al. 1989),

- (2) effective quantum yield

$$\Phi_{\text{PSII}} = F_M' - F_S / F_M'$$

(Genty et al. 1989),

- (3) photochemical quenching coefficient

$$qP = F_M' - F_S / F_M' - F_0'$$

(Krause and Weis 1991),

- (4) non-photochemical quenching

$$\text{NPQ} = F_M - F_M' / F_M'$$

(Müller et al. 2001), and

- (5) chlorophyll fluorescence decrease ratio

$$\text{Rfd} = F_P - F_S / F_S$$

(Lichtenthaler et al. 2005).

For definition of particular chlorophyll fluorescence levels entering the equations, *see* Supplementary Materials.

The treatment effect on photosynthetic processes was assessed by analysis of chlorophyll fluorescence transient (OJIP) by using the portable fluorometer FluorPen (Photon Systems Instruments, Czech Republic), particularly its shape and relative height of J/I chlorophyll fluorescence levels since they reflect consecutive process of induction of photosynthetic electron transport: OJ (so-called photochemical phase, from ~5–40 to 2–3 ms), JI (from 2–3 to 20–30 ms) and IP (from 20–30 to 100–300 ms). The O-J phase (the) depends on the intensity of the exciting light and is characterized by the reduction of the acceptor side of photosystem 2 (PSII): quinone acceptors Q_A and Q_B . Chlorophyll fluorescence rise subsequent to the J point is due to additional reduction of the acceptor side of PSII, the plastoquinone pool (PQ pool).

Leaf area and biomass development

Leaf area increase as dependent on the time of cultivation was calculated from the projected area of a leaf rosette for each individual plant and expressed as number of pixels taken by the rosette at each day of chlorophyll fluorescence measurements. Average leaf area was calculated as a mean of six replicates (plants) belonging

to a certain treatment. Average leaf area was plotted against the time of cultivation. Above-ground biomass was evaluated at the end of experiment (day 39) as a dry mass (DM) for particular treatment (six replicates per treatment) and the treatment-dependent differences in DM specified.

Leaf spectral properties

On Day 39, reflectance spectra were measured within the range of 680-800 nm using a Spectral Pen RP 400 spectroradiometer (Photon Systems Instruments, Brno, Czech Republic). Leaves were placed into a clip that enabled a constant distance from the instrument measuring head and

darkened for 5 min. Then, spectral reflectance were measured and the NDVI, OSAVI, ZMI, GM2 and RDVI indexes associated with leaf vitality evaluated using a PSI software version 1.1.0.21 (Photon Systems Instruments, Brno, Czech Republic).

Determination of heavy metals in samples

Lyophilized samples of *Arabidopsis thaliana* were analyzed in a trace laboratory according to the procedures published in Coufalík et al. (2024). At first, the samples were mineralized in ultrapure HNO₃ using an UltraWAVE microwave mineralizer (Milestone, Italy). Subsequently, the contents of Al, Cd, Cr, Cu, Ni, Pb, and Zn were determined using electrothermal

atomic absorption spectrometry (ETAAS). The conditions for the determination of Cd, Cr, Cu, Ni, Pb, and Zn using the Analyst 600 (PerkinElmer, USA) were published in Coufalík et al. (2024). The determination of Al in samples was performed using a standard analysis procedure (PerkinElmer, USA).

Statistical analysis and data processing

The experiment was conducted in a completely randomized design with six independent replicates per treatment. Treatment effects were analyzed using one-way or two-way analysis of variance (ANOVA) in Statistica, followed by

Tukey's post hoc test at a significance level of $p \leq 0.05$. Graphs output parameters related to plant growth and photosynthetic performance (chlorophyll fluorescence data) were generated using SigmaPlot v. 12.

Results

Plant growth

Leaf area (LA) development showed the positive effects of Hoagland solution (Fig. 1) compared to demineralized water. Similarly, addition *M. rubra* led to a positive effect on LA in a course of cultivation time (Figs. 1, 2). The most accelerated LA development was found for the H+B+M treatment, i.e. for the *A. thaliana* plants cultivated in regolith with addition of biochar, *M. rubra*, and supplies with the Hoagland solution. Treatment with exclu-

sive biochar addition, and the W+B+M treatment led to decrease LA development compared with the 'not biochar' treatments. LA development followed a *S-curve*, reaching LA maximum on Day 32 in vigorous treatments (Fig. 2, upper panel), and day 25 in less vigorous treatments (W, H, and W+B). Decline of LA values was apparent in the final part of cultivation time, most probably due to space limitation.

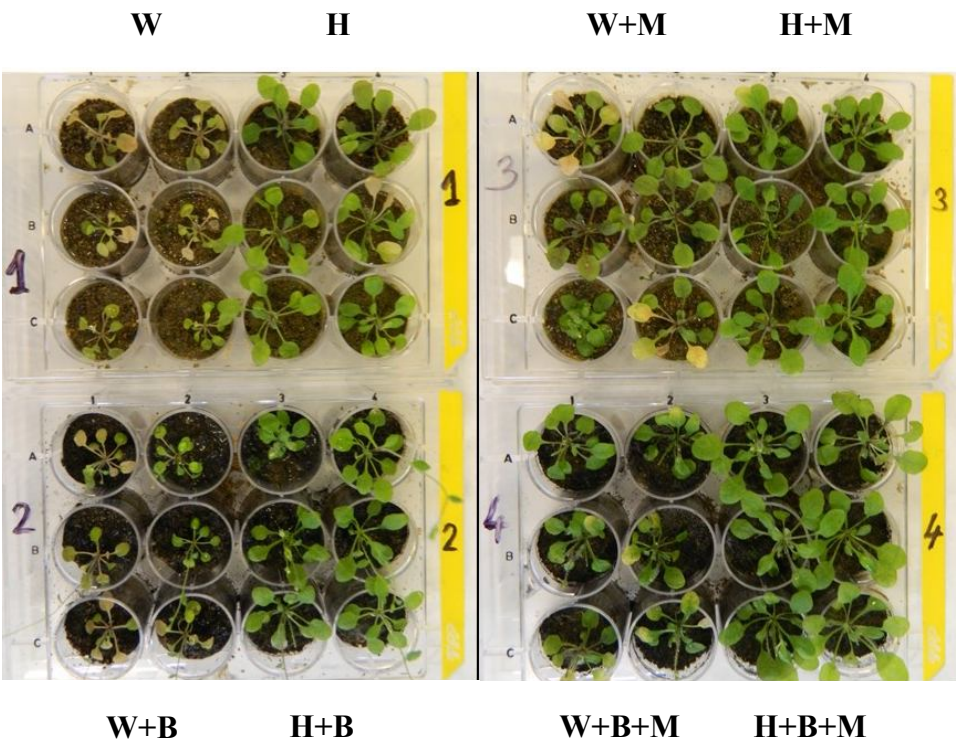


Fig. 1. *A. thaliana* plants on Day 32. Positive effects of Hoagland solution (compared to water) and *Mokoshia rubra* (here inscribed as *Massilia rubra*) addition are apparent.

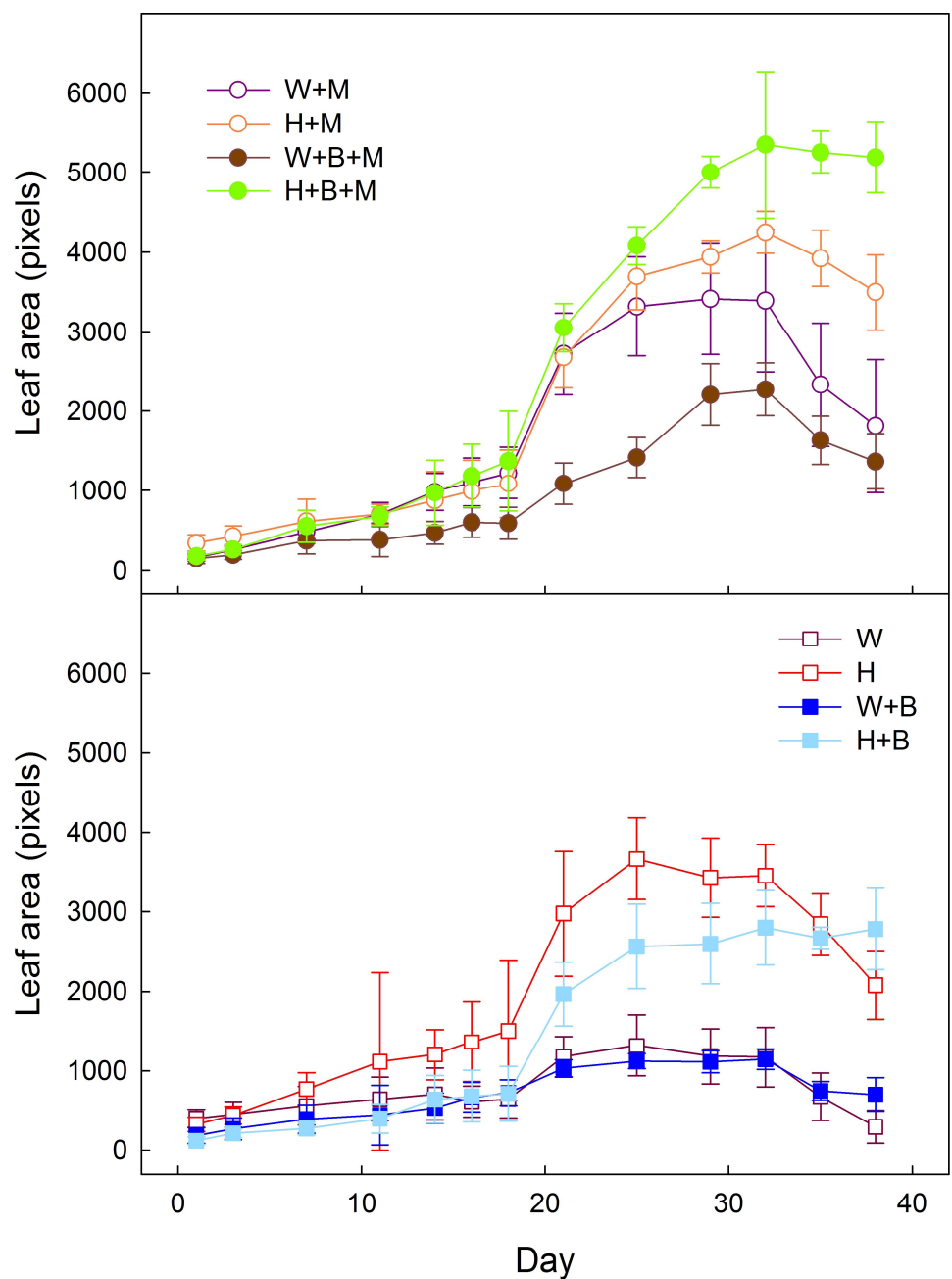


Fig. 2. Leaf area development in *A. thaliana* plants within the cultivation period. Positive effects of Hoagland solution (compared to water) and *Mokoshia rubra* addition are apparent.

Photosynthetic parameters (OJIP curves)

Fig. 3 shows the polyphasic increase in ChlF transient (OJIP curve) in plants exposed to individual experimental treatments. Low values and an overall flattening of the OJIP curve are evident for the water-treated plants, most apparently for the W+B+M, W+B, and W treatments. Conversely, the OJIP curves measured for the plants treated with Hoagland solution reached higher F_p values than those using demineralised water.

The normalized curves (F_p -normalized) presented in Fig. 4 show a significant difference in the J to P phase, namely in the elevated ChlF values for the variants irrigated with water alone. Higher ChlF values in the J–P phase indicate that the redox state of the PQ pool was negatively affected (*see* Discussion for further details). In the variants treated with *M. rubra*, no difference was apparent in the shape or course of the normalized OJIP curve.

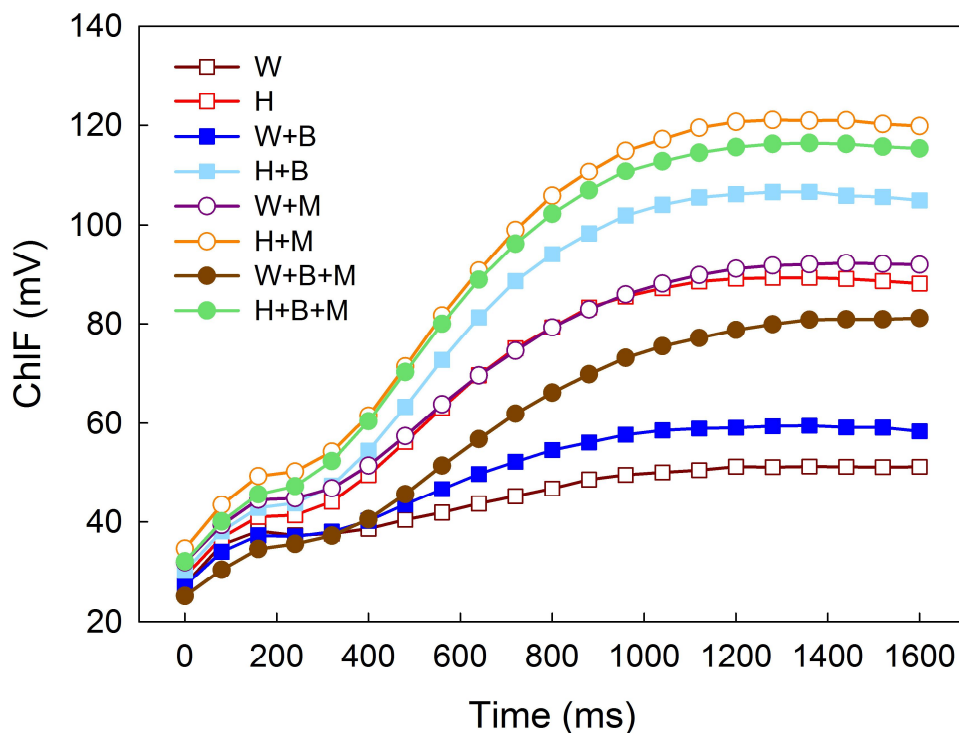


Fig. 3. Fast Chl fluorescence transient (absolute values) as recorded for particular treatments (lines and data points are means of 10 replicates). *Note:* dark red (W), light red (H), dark blue (W+B), light blue (H+B), dark pink (W+M), orange (H+M), brown (W+B+M), light green (H+B+M). *Key to the abbreviations:* B – biochar, M – Mokoshia, H – Hoagland solution, W – water.

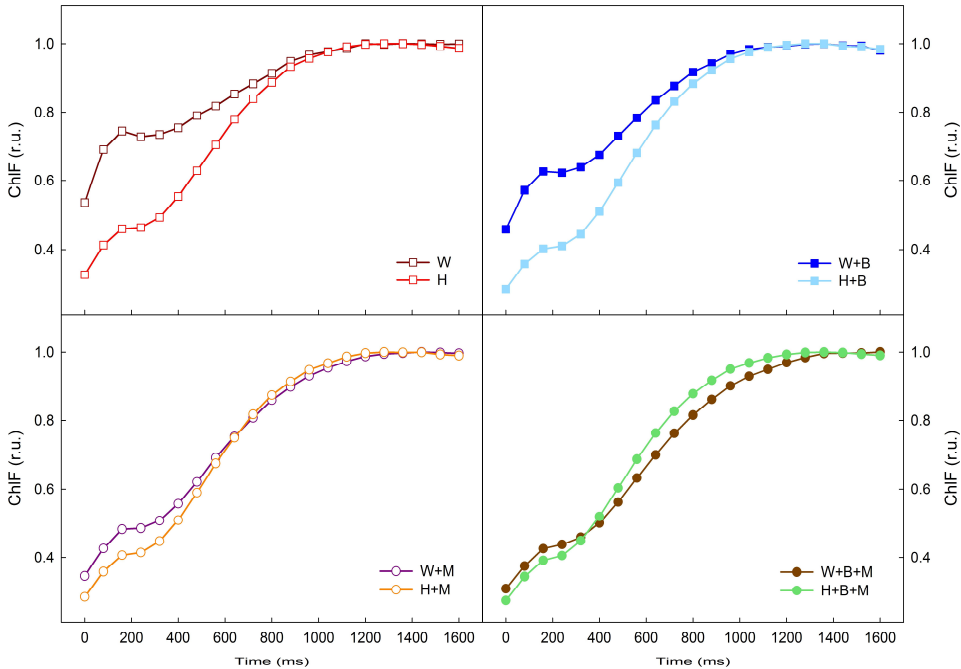


Fig. 4. Fast Chl fluorescence transient (normalized to F_p) as recorded for particular treatments and watering régime. The lines and data points are means of 10 replicates. Addition of *Mokoshia rubra* alleviates negative effect of watering by water. *Note:* dark red (W), light red (H), dark blue (W+B), light blue (H+B), dark pink (W+M), orange (H+M), brown (W+B+M), light green (H+B+M).

Irrespective of treatment, maximum yield of PSII (F_v/F_m) showed an increase with the cultivation time (Fig. 5). On Day 39, positive effect of the H+B, H+B+M and W+B+M treatments was apparent while the other treatments showed somewhat lower F_v/F_m values. Most negative effect was found for F_v/F_m on Day 39 for the W+B, and W treatments. The same two treatments had negative effect on Φ_{PSII} , and Rfd as well. The decline of Rfd values

found at the end of cultivation period was fast and pronounced. The Rfd reached extremely low value, close to initial ones, at the end of cultivation period indicating a drop in overall physiological functioning of the *A. thaliana* plants and their low vitality. The most positive effect on Rfd was apparent for the plants treated with Hoagland solution, *i.e.* (H, H+B, H+M, and H+B+M).

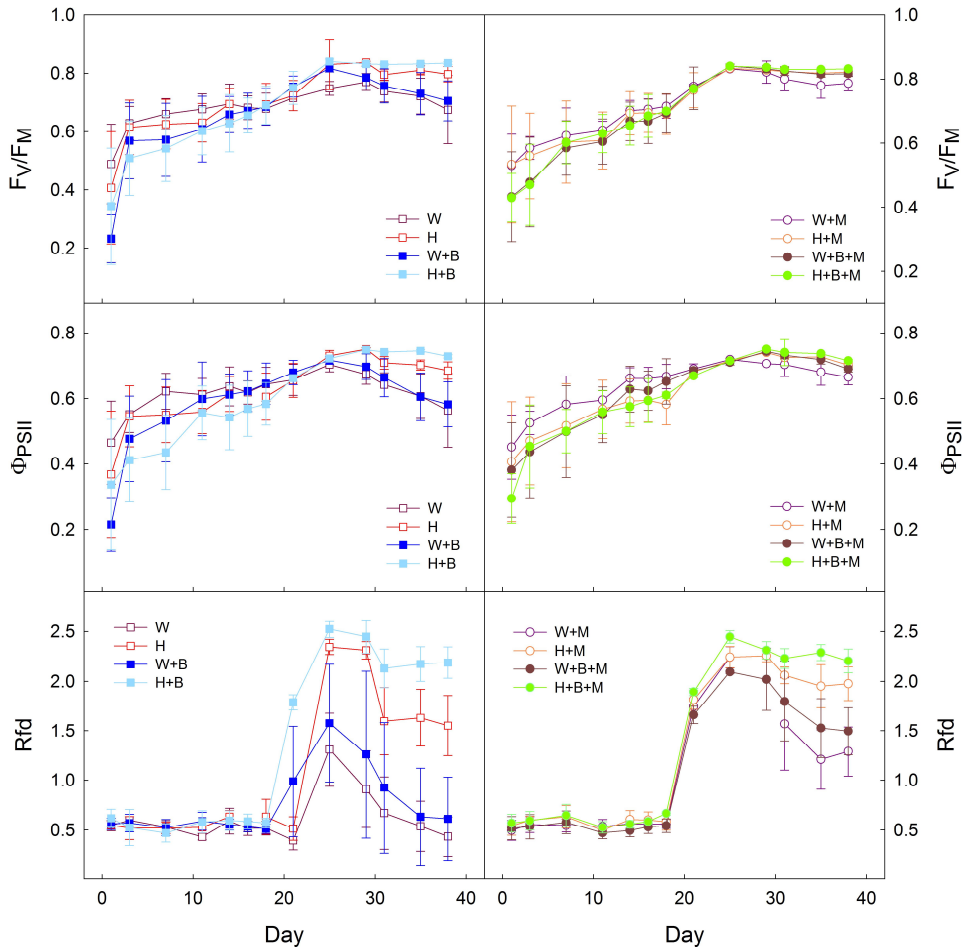


Fig. 5. Time courses of chlorophyll fluorescence parameters (F_v/F_m , Φ_{PSII} , and R_{fd}) recorded in *A. thaliana* plants for particular cultivation treatments. Data points are means of six replicates (plants).

Spectral reflectance indices (NDVI, OSAVI, ZMI, GM2, and RDVI) measured on Day 39 showed positive effects of Hoagland solution and the addition of *M. rubra* into the regolith at the Day 0. The data showed that the five spectral reflectance indices might be used as indicators of vitality of the experimental plants.

The determined contents of Al, Cd, Cr, Cu, Ni, Pb, and Zn in *A. thaliana* leaf biomass are presented in Fig. 8. The limits of detection (LOD) and quantification (LOQ) were calculated as three and ten times the

standard deviation of blanks, respectively. Compared to the watered regolith treatment, majority of additions (biochar, *M. rubra*, Hoagland solution treatments) showed an effect on Al, and Ni ions content in the leaves. The additions led to their substantial decrease. Content of Cd, Cu, Pd, and Zn was either treatment-independent or showed a slight increase in Hoagland, *M. rubra*, and their combination treatments. The increase was, however, at the edge of statistical significance.

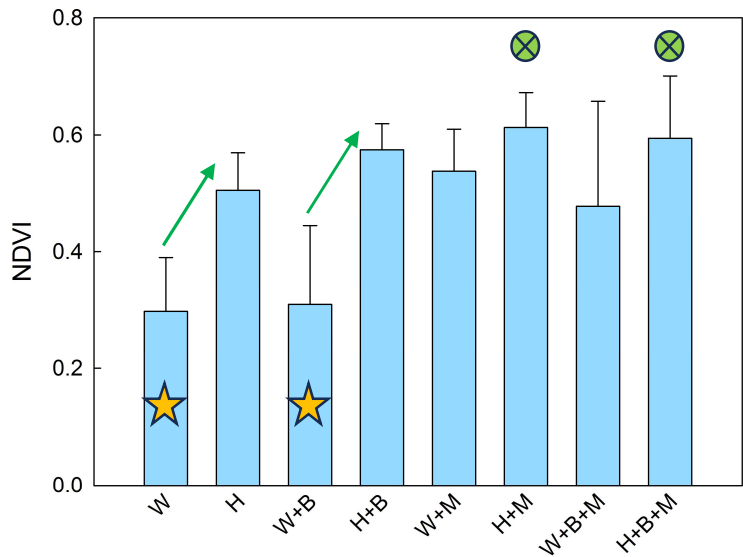


Fig. 6. The effect of particular treatment on normalized difference vegetation index (NDVI) of *Arabidopsis thaliana* plants cultivated for 38 days. Negative effect of watering by demineralized water is indicated by an asterisk. Positive effect of addition of *Mokoshia rubra* is indicated by ⊗. The effect of Hoagland solution (compared by watering by demineralized water) is indicated by an arrow.

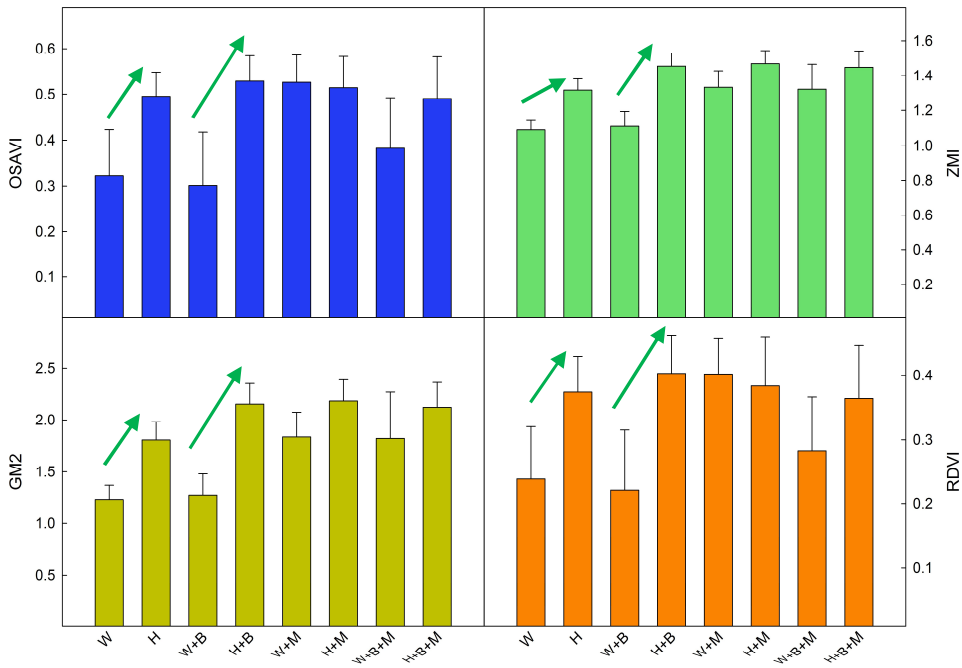


Fig. 7. The effect of particular treatments on OSAVI, ZMI, GM2, and RDVI vegetation indices in *Arabidopsis thaliana* plants cultivated for 39 days. The positive effect of Hoagland solution (compared by watering by demineralized water) is seen and indicated by an arrow.

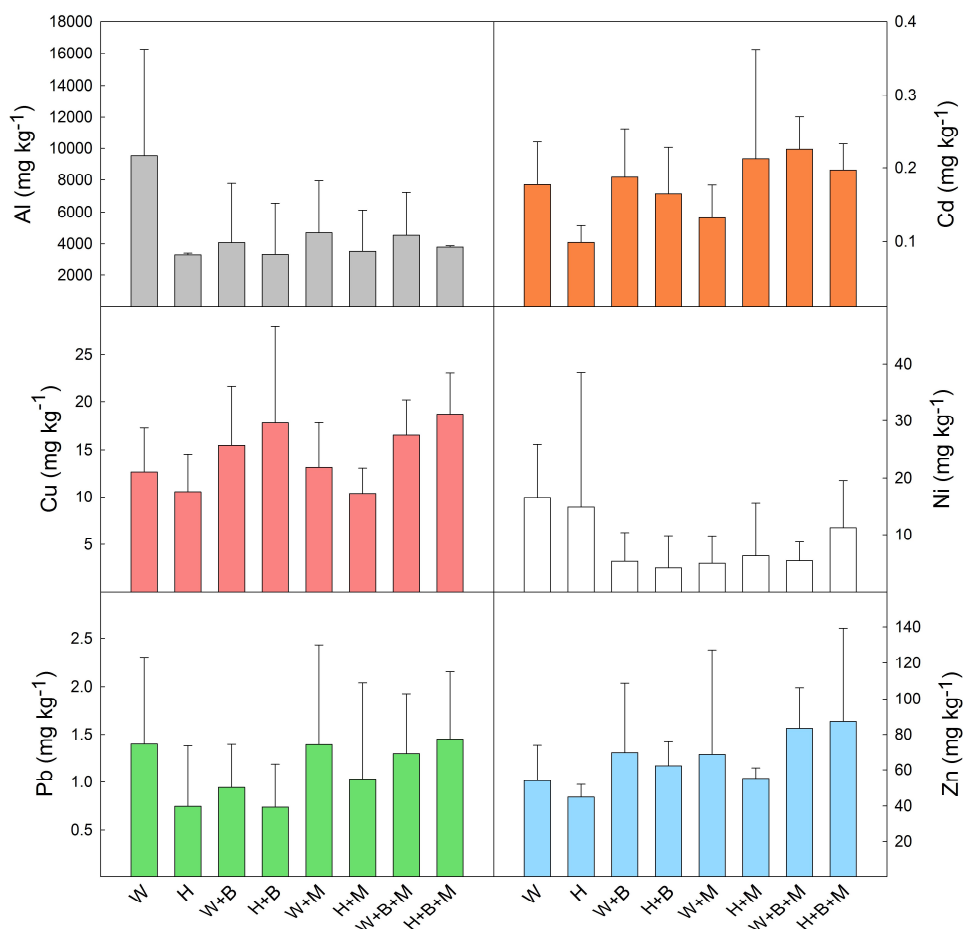


Fig. 8. Contents of heavy metals in the above-ground biomass (leaves) in *A. thaliana* plants cultivated in the experimental treatments.

Discussion

Plants grown in regolith *per se* (demineralized water, *see* Fig. 1) showed remarkable growth reduction apparent after two weeks of cultivation. This is well comparable to the data presented by Paul et al. (2022) for the lunar regolith. The study reports slowed plant growth and development with signs of severe stress morphologies. The *A. thaliana* germinating in several regoliths (Apollo 11, 12, 17, and JSC-A1 simulant) exhibited reactive oxygen species formation and the responses

similar to salt stress. The study found that *A. thaliana* grown in lunar regolith differentially expressed genes indicating ionic stresses. In our study, negative effect of demineralized water was (compared to Hoagland solution treatment) was found indicating ion imbalance in the plants exposed to W, and W+B treatments.

Irrespective of treatment, watering by the Hoagland nutrient solution apparently improved growth, demonstrating that nutrient availability is a driving factor of plant

growth in regolith-based substrates. This is well comparable to the study of Caporale *et al.* (2023). Recent study of D'Annunzio *et al.* (2026) provided novel insights into the effects of lunar regolith on plant growth by analyzing differential gene correlations in *Arabidopsis thaliana* grown in Apollo 12 and Apollo 17 regolith compared to simulants. While the study reports different negative consequences for the Apollo 12 (extreme functional phytotoxic effects, disrupting key genes involved in reproductive development, cell wall remodelling, and metal homeostasis), and 17 (nutrient limitations, particularly affecting iron and phosphate metabolism) regolith, it concludes that lunar regolith, when appropriately supplemented with nutrients, could potentially support plant cultivation in extraterrestrial environments. This fits our findings reported for *A. thaliana* treated in Antarctic regolith. Recent studies focused on *Arabidopsis thaliana* growth in Lunar regolith and/or its analog revealed that plants can germinate and grow but show irregularities in vegetative development and activate a variety of stress response pathways in response to oxidative stress. Study of Barcenilla *et al.* (2024) revealed oxidative damage in telomeres of *A. thaliana* grown in a lunar regolith simulant causing changes in telomere dynamics, telomerase enzyme activity and genome oxidation. The study also reports positive effects of antioxidants on *A. thaliana* growth and seed production if the lunar regolith simulant is pre-washed with an antioxidant cocktail.

Our study showed growth suppression in the plants grown in the regolith, and supplied by water. This is well consistent with recent study of Handy *et al.* (2026) reporting negative impact of Lunar regolith simulant on potato plant size and tuber yield. In our study, the flattening of OJIP curves (Fig. 3) in the treatments using demineralized water could be a consequence of heavy metal negative effects on PSII, particularly Al, Cd, and Pb (*see* Fig. 8).

Heavy metal induced OJIP flattening reflects structural and functional damage to PSII, including the inactivation of reaction centers and the inhibition of the oxygen-evolving complex (OEC) – Giordano *et al.* (2025). These heavy metal ions-induced changes are typically associated with the photosynthetic electron transport chain, specifically preventing proper reduction of the quinone acceptor and altered redox state of plastoquinone pools.

The absence of nutrient from the deionized water was the main factor determining the loss of photosynthetic functions, as well as reduction in overall vitality (*see* NDVI, Fig. 6). However, the addition of *Massilia* culture itself improved significantly the *A. thaliana* growth performance, proving its potential for crop cultivation in Antarctic regolith. The spectral reflectance indices used in our study are sensitive to stress in plants since OSAVI is typically used for estimating plant chlorophyll content, detecting vegetation stress, and monitoring plant health. ZMI and GM2 are generally sensitive to stress (Rosa *et al.* 2023). RDVI provides insights into vegetation health, density, and chlorophyll content in plants and their photosynthetic organs.

Moreover, the use of non-invasive tools such as ChlF and spectral reflectance confirmed their utility for a fast and non-destructive phenotyping, allowing a complete evaluation of plant's photosynthetic performances – and health status – right on the spot. However, several limitations remain: the large scale application of ChlF as well as spectral reflectance, especially in an agronomic scenario far from controlled laboratory conditions – should be complemented with other metrics (such as net photosynthesis/gas exchange metrics or biometrics) (Haq *et al.* 2026a, b).

Inoculation of the regolith with *M. rubra* significantly improved *A. thaliana* above-ground biomass, and photosynthetic efficiency of PSII which is comparable to the effects reported by Haq *et al.* (2025) for *Nicotiana tabacum* and *N. benthamiana*.

treated with the same bacterial strain. *M. rubra* has been tested and proved as a good PGPB for stress mitigation; however, this specific strain lacks a deeper traits analysis, and plant-bacteria interaction; therefore, future studies will need to focus on the mechanisms behind the activity of *M. rubra* as a PGPB and to investigate whether extracts from this bacteria strain may determine a resource for crop cultivation. Atkin et al. (2025) reported positive effects of addition of *Mesorhizobium ciceri*, a gram-negative, nitrogen-fixing motile bacteria, and arbuscular mycorrhizal fungi consortium to a lunar regolith simulant on both above- and below-ground biomass, suggesting potential for biological nitrogen fixation. Biochar addition had less effect than *M. rubra*, however, follow-up studies should address different shares of biochar in order to optimize substrate properties (Ni et al. 2018, Adhikari et al.

2022, Rombel et al. 2022, Ng et al. 2025, Mei et al. 2025).

Lunar regolith tends to be alkaline, with pH levels typically ranging from 7.5 to 9.0. This alkalinity can affect nutrient availability and plant growth, requiring potential amendments to optimize conditions for farming. Some alterations in pH values might be expected during the cultivation period as a consequence of watering (demineralized water versus Hoagland solution). In our experiment, addition of biochar may cause further alkalinization of the Antarctic regolith, and possible restriction of element solubility and bioavailability of *A. thaliana* roots. In conclusion, biochar amendment in lunar regoliths and/or lunar regolith simulants needs some organic matter to improve growth substrate characteristics and stabilization of heavy metal allocation in soil (Radziemska et al. 2023).

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Web sources / Other sources

- [1] Czech Collection of Microorganisms (Brno, Czech Republic): <http://www.sci.muni.cz/ccm/>

Supplementary Materials

AGK-2010	Poland	2013	FJS-2	Japan	1998
ALRS-1	Australia	2012	FJS-3	Japan	1998
ALS	USA	1993	FROST-Y	USA	—
BH-1	China	2021	GCA-1	USA	2008
BH-2	China	2021	GRC-1	USA	2010
BHLD20	China	2017	GRC-3	USA	2013
BP-1	USA	2010	GreenSpar	USA	2020
CAS-1	China	2009	GSC-1	USA	2008
CLDS-i	China	2017	HIT-LRS-1	China	—
CLRS-1	China	2009	HR-2	UK	—
CLRS-2	China	2009	HUST-1	China	2022
CMU-1	USA	—	CHENOBI	Canada	2009
CQU-1	China	—	IGG-01	China	—
CSM-CL	USA	2010	IRSM-1	China	—
CSM-CL-S	USA	2010	JLU-H	China	2022
CSM-LHT-1	USA	2021	JSC-1	USA	1994
CSM-LHT-1G	USA	2023	JSC-1A	USA	2005
CSM-LMT-1	USA	2021	JSC-1AC	USA	2005
CUG-1	China	2010	JSC-1AF	USA	2005
CUG-1A	China	2010	JSC-2	USA	—
CUMT-1	China	2022	JSC-2A	USA	—
DNA-1	Italy	2014	KAUMLS	South Korea	2014
DNA-1A	Italy	2019	KIGAM-L1	South Korea	—
DUST-Y	USA	2019	KLS-1	South Korea	2018
EAC-1	Germany (ESA)	2017	KOHLs-1	South Korea	2014
EAC-1A	Germany (ESA)	2020	Kohyama Simulant	Japan	2008
EB-2	UK	—	LBD	China	—
ES-1	UK	2014	LCATS-1	USA	2020
ES-2	UK	2014	LHA-1	USA	2020
ES-3	UK	2014	LHD-1D	USA	2024
ES-4	UK	2014	LHS-1	USA	2020
FEFU-1	Russia	—	LHS-1-25A	USA	—
FJS-1	Japan	1998	LHS-1D	USA	2021
FJS-1g	Japan	—			

LHS-1E	USA	2025	NU-LHT-2C	USA	2008
LHS-2	USA	2025	NU-LHT-2E	USA	—
LHS-2E	USA	2025	NU-LHT-2EG	USA	—
LMA-1	USA	2020	NU-LHT-2M	USA	2008
LMS-1	USA	2020	NU-LHT-3M	USA	—
LMS-1D	USA	2021	NU-LHT-4M	USA	2020
LMS-1E	USA	2025	NU-LHT-5M	USA	2022
LMS-2	USA	2025	NUW-LHT-5M	USA	2024
LSP-2	USA	2025	OB-1	Canada	2007
LSS-1	USA	1968	OB-1A	Canada	2008
LSS-2	USA	1968	OPR	USA	—
LSS-3	USA	1968	Agglutinate		
LSS-4	USA	1968	OPRFLCROSS1	USA	2019
LSS-5	USA	1968	OPRFLCROSS2	USA	2019
LSS-ISAC-1	India	2020	OPRH2N	USA	2010
LuNOR	Norway	—	OPRH2W	USA	2010
LuSIC-1	Spain	2025	OPRH3N	USA	2010
LX-I50	Germany	2025	OPRH3W	USA	2010
LX-M100	Germany	2025	OPRH4N	USA	2010
LX-T100	Germany	2025	OPRH4W30	USA	2010
LZS-1	Spain	2022	OPRL2N	USA	2010
Maryland-Sanders	USA	—	OPRL2NT	USA	2010
MKS-1	Japan	1994	OPRL2W	USA	2010
MLS-1	USA	1990	Oshima		
MLS-1A	USA	—	Simulant	Japan	2008
MLS-1P	USA	1990	OUEB-1	UK	—
MLS-2	USA	1992	OUHR-1	UK	—
Mooncastle	USA	—	OUSR-1	UK	—
NAO-1	China	2008	PolyU-1	China	2024
NAO-2	China	—	QH-E	China	—
NEU-1	China	2019	SCC-1	UK	—
NEU-1A	China	2019	SCC-2	UK	—
NEU-1B	China	2019	SR-2	UK	—
NJU-1	China	—	TBG-1	Turkey	—
NU-LHT	USA	2007	TJ-1	China	2012
NU-LHT-1D	USA	2007	TJ-2	China	2010
NU-LHT-1M	USA	2007	TLH-0	Spain	—
			TLM-0	Spain	—
			TLS-01	Thailand	2021

TLS-01A	Thailand	2023	TYII-0	China	—
TRI-1	India	—	TYII-1	China	—
TUBS-H	Germany	—	TYII-2	China	—
TUBS-I	Germany	2022	UoM-B	UK	2020
TUBS-M	Germany	2020	UoM-W	UK	2020
TUBS-T	Germany	2020			

Table 1S. List of Lunar regolith simulants. Adopted from the database of the Spring Institute for Forest on the Moon (<https://thespringinstitute.com/>).

Hoagland solution composition

Macronutrients:

- KNO_3 (Potassium Nitrate): 6.0 mM
- $\text{Ca}(\text{NO}_3)_2$ (Calcium Nitrate): 4.0 mM
- $\text{NH}_4\text{H}_2\text{PO}_4$ (Ammonium Dihydrogen Phosphate): 2.0 mM
- $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ (Magnesium Sulfate): 1.0 mM

Micronutrients:

- H_3BO_3 (Boric Acid): 46.0 μM
- $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$ (Manganese Chloride): 9.0 μM
- $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ (Zinc Sulfate): 0.8 μM
- $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ (Copper Sulfate): 0.3 μM
- H_2MoO_4 (Molybdic Acid) or $\text{Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O}$: 0.1 μM

Iron Source:

- Fe-EDTA (or Fe-DTPA): 5.0 μM to 10.0 μM (Chelated iron).
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Table 2S. Composition of Hoagland solution, a standard liquid medium used for growth of plants in hydroponics and similar cultivation systems.