

The Artemis I mission reshaped seed amino acid composition and post-flight plant growth in a genotype-dependent manner

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Abstract

Plants will be critical to sustainable life support during long-duration space exploration, yet the effects of deep-space exposure on seed metabolism and plant growth remain unclear. Using *Arabidopsis thaliana* germplasm with altered branched-chain amino acid (BCAA) biosynthesis, we examined the impact of the Artemis I mission environment on seed amino acid composition, viability, and post-flight plant performance. We found that the Artemis I mission induced genotype-dependent change in seed free amino acid profiles, with a BCAA-enriched mutant, *ipms1*, showing increased levels of essential amino acids, whereas wild type and other BCAA-altered mutants, *ahass2* and *omr1*, were largely unaffected. Despite these metabolic changes, seed viability and germination efficiency were preserved across all genotypes. In contrast, the Artemis I mission triggered pronounced, genotype-specific alterations in free amino acid homeostasis during early seedling development, and it promoted enhanced rosette growth in wild type and *ipms1*, but not in *ahass2* or *omr1*. Together, these findings identify BCAA metabolism as an important factor influencing plant metabolic and growth responses related to deep-space exposure conditions, with implications for germplasm selection in future lunar and Mars missions.

Introduction

Flowering plants propagate primarily through seeds, which serve as compact nutritional storehouses essential for both seedling establishment and human nutrition. Seeds accumulate carbohydrates (e.g., starch), storage proteins, and lipids that support embryo survival during dormancy and fuel early post-germinative growth. During the initial stages of germination, these stored reserves provide the energy needed for heterotrophic seedling development before photosynthesis is initiated. Beyond their role in plant reproduction, seed-derived foods supply ~70% of the global human caloric intake and provide essential nutrients for human health¹.

Seeds provide a practical form for transporting live plant material during deep-space transit because they have low mass and volume, can be stored for long-term, and require minimal maintenance compared to seedlings and mature plants. Since the initiation of plant research in space, considerable progress has been made in understanding how microgravity and spaceflight conditions influence seed storage, germination, and early development in low Earth orbit (LEO)^{2–10}. However, much less is known about how seeds and subsequent plant growth respond to environmental conditions encountered beyond LEO. This knowledge gap is particularly important for implementing plant-based systems during deep-space exploration, where maintaining seed quality and supporting reliable germination and healthy, nutritious plant growth will be essential for both transit and environments relevant to the Moon or Mars^{2–10}.

Spaceflight environment exposes biological systems to a unique combination of stressors, including ionizing radiation, altered gravity, temperature fluctuations, and extended storage under constrained environmental control^{11, 12}. These factors are known to disrupt central metabolic pathways, including amino acid homeostasis, which are critical for cellular maintenance, stress tolerance, and growth^{13–18}.

Amino acids play key roles not only as protein building blocks but also as metabolic regulators, redox buffers, and precursors for signaling molecules^{19, 20}. In plants, amino acid composition at the seed stage can influence stress resilience during dormancy and determine metabolic capacity during early post-germinative growth, a period that is particularly sensitive to environmental perturbations^{21, 22}. Despite the central importance of amino acids to plant physiology and human dietary value, relatively little is known about how exposure beyond LEO alters amino acid pools in seeds or how such changes influence subsequent plant performance. The Artemis I mission provided a unique opportunity to begin addressing this gap.

Among the amino acids of particular interest in this context are the branched-chain amino acids (BCAAs; isoleucine, leucine, and valine), which are important for plant development and nutritional quality. Because humans do not synthesize these essential amino acids, they must be obtained from plant-derived dietary sources. In plants, the productivity of the BCAA biosynthetic pathway is controlled by the activity of a small number of key enzymes, including isopropylmalate synthase 1 (IPMS1), threonine deaminase (also known as L-O-methylthreonine-resistant 1, OMR1), and acetohydroxyacid synthase (AHAS)²³. These enzymes catalyze committed steps in BCAA biosynthesis from threonine and pyruvate. While strong loss-of-function mutations in *IPMS1*, *AHAS*, and *OMR1* disrupt overall amino acid homeostasis, leading to widespread metabolic rearrangement *in planta*^{20, 23, 24}, specific mutant alleles of these enzymes exhibit selective overaccumulation of individual BCAAs relative to WT with minimal effects on the broader amino acid profile. In particular, a point mutation in the *Arabidopsis* *IPMS1* gene, *ipms1-1^D* (hereafter referred to as *ipms1*) shows elevated leucine (Leu) levels; the *AHAS* small subunit mutant *ahass2-1^D* (hereafter *ahass2*) accumulates higher levels of valine (Val) and Leu; and the OMR1 allosteric domain mutant *omr1-11^D* (hereafter *omr1*) display pronounced increase in isoleucine (Ile) BCAAs relative to wild type (WT)²³. These genetically defined differences make this set of mutants particularly informative for dissecting the role of specific BCAAs in seed metabolic stability, stress responses, and plant growth following Artemis I mission exposure. Artemis I was NASA's first mission in its Artemis program, aimed at returning humans to the Moon and paving the way for future missions to Mars. Launched in November 2022, it was an uncrewed test flight of the Space Launch System rocket and the Orion spacecraft. The mission traveled beyond the Moon, entering a distant retrograde orbit before safely returning to Earth, validating key systems such as navigation, heat shielding, and reentry. It also carried live experiments, including biological materials, to examine how deep-space conditions affect living materials. Artemis I marked a significant step toward upcoming crewed missions and renewed lunar exploration.

In this study, we aimed to establish foundational knowledge necessary to mitigate the negative impacts of the Artemis I mission on seeds and improve strategies for seed transportation and germplasm selection for future deep space exploration. Emphasis was placed on defining how the exposure to the Artemis I lunar mission environment influenced amino acid homeostasis in seeds and how these metabolic changes related to seed viability, early developmental robustness, and subsequent vigor of germinated plants. By combining the unique environment of the Artemis I lunar mission with genetically

defined *Arabidopsis* lines exhibiting elevated levels of specific BCAAs, we directly tested whether metabolic composition shapes plant responses to the Artemis I mission environment. This approach enabled the identification of metabolic traits that support seed resilience and growth plasticity following the Artemis I mission and provides a platform for leveraging targeted nutritional and metabolic characteristics in the design of plant systems for long-duration lunar and Mars exploration.

Results

Environmental conditions experienced by plant samples during the Artemis I mission

To characterize the environmental conditions experienced by the BCAA-enriched seeds during the Artemis I mission, temperature and relative humidity (RH) were continuously monitored throughout the Artemis-I mission using environmental data loggers and radiation exposure was quantified by two types detectors: crew active dosimeters (CAD) and radiation area monitors (RAM) within each container position of Science Bag 1, which was stowed in a container assembly mounted to the Orion backbone (Fig. 1a-c). Environmental data was collected upon return of the samples to the Kennedy Space Center. The temperatures recorded during flight ranged between 53 and 78.8°F. The upper and lower data sensors showed nearly identical profiles, indicating there was minimal spatial variation within the science bag (Fig. 1d, Supplemental Data 1).

Following launch on 16 November 2022, temperatures were initially elevated (71.6–78.8°F) and gradually declined with intermittent fluctuations during transit through Earth's orbit. As Orion approached its first lunar flyby in mid-November, temperatures decreased sharply between 53.6 to 57.2°F and remained low for several days with minor oscillations. In early December, temperatures partially recovered to intermediate values (62.6 to 68.0°F), followed by additional transient cooling events and a second pronounced temperature decrease in mid-December, reaching the lowest recorded values (51.8 to 55.4°F), before rising rapidly to above 68°F toward the end of the mission. Overall, temperatures remained within a moderate range throughout the mission with no prolonged exposure to high-temperature conditions.

In contrast to temperature, relative humidity (RH) showed a rapid decline early in the mission, followed by a prolonged low-humidity phase and a sharp increase near the end of flight (Fig. 1e, Supplemental Data 1). At launch, RH values were relatively high (45 to 55%) but dropped within the first several days to a stable low range of approximately 18 to 22% RH, where the RH remained for most of the mission. Only minor fluctuations were observed during transit. Near landing in mid-December, RH increased rapidly, reaching approximately 40 to 50% RH before the conclusion of the flight. Measurements from the upper and lower data loggers were highly similar for both temperature and RH, indicating minimal microenvironmental stratification within the sample enclosure. Overall, seeds experienced predominantly low-humidity conditions throughout the mission, with only brief periods of elevated RH at the beginning and end of the flight.

Consistent with the environmental data, radiation exposure was comparable between upper and lower sample positions. Total mission radiation doses were 11.7 ± 0.4 mGy and 11.4 ± 0.4 mGy for the upper and lower positions, respectively, while crew active dosimeter (CAD) Artemis I mission doses were 12.1 ± 0.5 and 12.6 ± 0.5 mGy. Based on CAD measurements, cumulative absorbed dose in the flight samples was ~ 25-26-fold higher than in the post flight control samples (Table 1, Supplemental Data 2).

Effects of deep-space travel on amino acid composition in BCAA-enriched seeds

To monitor the impact of deep-space flight on the seed amino acid composition, free amino acid (FAA) abundances were quantified in dry *A. thaliana* seeds of WT (Col-0) and the BCAA-enriched mutants *ipms1*, *ahass2*, and *omr1* from Earth synchronous ground control (SGC) and Artemis I mission (Flight) samples (Fig. 2a). To do this, we used liquid chromatography-tandem mass spectrometry to quantify the abundance of FAAs, excluding cysteine due to technical limitations, from dry seed.

The SGC dry seeds of *ipms1*, *ahass2*, and *omr1* mutants showed an increase in BCAA abundance compared to Col-0 (Supplemental Data 4). These results agree with previous seed profiling results for these genotypes²³. Compared to Col-0, dry seeds of SGC *ipms1* showed an increase in Leu and Ile, with fold changes of 1.30 and 1.17, respectively, accompanied by a reduction in Val (0.57-fold change). In contrast, *ahass2* and *omr1* SGC dry seed samples exhibited an increase in Leu (3.21 and 7.98-fold change respectfully), Ile (2.52 and 20.01-fold change respectfully) and Val (3.23 and 6.59-fold change respectfully). Moreover, we observed that several other amino acids were altered in the mutants relative to Col-0, contrary to previous reports^{20, 23}. These data suggest that the three genotypes used in this study exhibit distinct baseline metabolic states at the dry seed stage on Earth.

Quantification from Flight seed samples (Fig. 2a, Supplemental Data 3) revealed that FAA levels in Col-0, *ahass2*, and *omr1* dry seeds were not significantly altered by Artemis I mission conditions (Fig. 2b, d, e). In contrast, *ipms1* seeds exhibited significant changes in FAA composition following Flight (Fig. 2c). Of the 19 amino acids measured, the levels of Leu, Ile, Val, histidine (His), lysine (Lys), methionine (Met), phenylalanine (Phe), threonine (Thr), asparagine (Asn), and glutamate (Glu) significantly increased in Artemis I mission-exposed *ipms1* seeds, whereas aspartate (Asp) was reduced compared to Col-0 (Fig. 2c). Notably, essential amino acids, including the BCAAs, were elevated after Artemis I mission exposure (Fig. 2), resulting in an overall increase in total BCAA content relative to SGC control (Supplemental Fig. 1). Together, these results demonstrate the Artemis I mission environment drives genotype-dependent remodeling of seed free amino acid composition, with the BCAA-enriched genotype *ipms1* being the only genotype to exhibit statistically significant changes relative to its ground control.

Impact of the Artemis I mission on early seedling physiology of BCAA-enriched seeds

Because the Artemis I mission environment was expected to be suboptimal for seed storage and negatively affect post-germination performance, we hypothesized that BCAA-enriched seeds, which contain elevated levels of essential amino acids relative to WT, would better withstand Artemis I mission-associated stresses. To test this, we assessed seed viability and germination levels of Col-0 and the BCAA-enriched mutant seeds exposed to SGC or Flight conditions in long-day sterile culture conditions (Fig. 3a, Supplemental Data 5). After 5 days of growth, visual inspection of seedlings revealed no obvious morphological abnormalities across genotypes or treatments (Fig. 3b). Germination rates (defined by root emergence) and viability rate (determined by proper expansion of greened cotyledons) were then quantified.

Quantitative analysis showed high viability across all genotypes, with no statistically significant differences between SGC and Flight conditions (Fig. 3c). Similarly, germination rates were comparable between treatments for Col-0 and the mutant lines, indicating that exposure to the Artemis I mission environment did not impair viability (Fig. 3d). Together, these results indicate that seed viability and early post-germination growth are largely resilient to the conditions experienced during the Artemis I lunar mission conditions across all genotypes tested.

Effects of the Artemis I mission on amino acid homeostasis during early seedling development

Heterotrophic seedlings rely almost exclusively on stored seed nutrient reserves to drive hypocotyl elongation and establish photosynthetic capacity after reaching the light²⁵. Therefore, to further examine whether changes in seed nutrient composition associated with the Artemis I mission affect early seedling development, we analyzed dark-grown seedlings. Because the mutant seeds contain elevated levels of essential amino acids, we expected that Artemis I mission-associated effects would be most pronounced during this early stage of growth. To test this hypothesis, we assessed the effect of the mission environment on heterotrophic growth by measuring the extent of hypocotyl elongation of dark grown seedlings (Supplemental Fig. 2, Supplemental Data 6). WT and *ipms1* showed no significant changes in hypocotyl length following the Artemis 1 mission, whereas *ahass2* and *omr1* displayed significantly longer hypocotyl than their respective SGC controls (*ahass2*, $P < 0.01$; *omr1*, $P = 0.01$; Supplemental Fig. 2; Supplemental Data 6). These results indicate that hypocotyl growth responses to the Artemis I lunar mission are genotype dependent, with *ahass2* and *omr1* exhibiting longer hypocotyls than their respective SGC controls, whereas WT and *ipms1* showed no significant change.

Compared to Col-0, dry seeds of SGC *ipms1* showed an increase in Leu and Ile, with fold changes of 1.30 and 1.17, respectively, accompanied by a reduction in Val (0.57-fold change). In contrast, *ahass2* and *omr1* SGC dry seed samples exhibited an increase in Leu (3.21 and 7.98-fold change respectfully), Ile (2.52 and 20.01-fold change respectfully) and Val (3.23 and 6.59-fold change respectfully). Moreover, we observed that several other amino acids were altered in the mutants relative to Col-0, contrary to previous reports^{20, 23}.

Because hypocotyl elongation during heterotrophic growth depends on stored seed reserves, we next quantified FAA in the dark-grown seedlings to determine whether the Artemis I mission environment altered nutrient utilization during early seedling development. First, we examined dark-grown seedlings of *ahass2*, *ipms1*, and *omr1* from SGC dry seeds and found that mutants accumulated higher levels of certain BCAAs than WT (Fig. 4a, Supplemental Data 7 and 8). While amino acid profiles in dark-grown seedlings have not been previously reported for these mutants, the elevated BCAA levels observed in this study agree with trends previously described in dry seeds and seedlings^{20, 23}. Furthermore, post-hoc comparisons revealed that, compared to SGC, dark grown seedlings of from Flight samples had significantly decreased total amino acid levels in *ahass2* (p.adj = 0.002) and *omr1* (p.adj = 4.9×10^{-5}), while no significant changes were observed in Col-0 or *ipms1* (Supplementary Fig. 3, Supplementary Data 9).

Individual FAA abundances were then quantified in dark-grown seedlings derived from SGC and Flight samples (Fig. 4b-e). FAA quantification revealed that in Col-0 and *ipms1* samples, FAA abundancies were not significantly altered by the Artemis I mission (Fig. 4b,c). In contrast, dark-grown *ahass2* and *omr1* seedlings exhibited significant changes in FAA composition when grown from seeds that experienced the Artemis I mission environment (Fig. 4d,e). Of the 19 amino acids measured, Ile, Arg, His, Lys, Phe, Trp, and Tyr significantly increased, whereas Leu, Val, Met, Thr, Ala, Asn, Asp, Gln, Glu, Gly, Pro, and Ser significantly decreased in seedlings derived from *ahass2* seeds exposed to the Artemis I mission compared to SGC (Fig. 4d). For dark grown seedlings derived from *omr1* Artemis I mission seeds compared to SGC seeds, a significant increase in Arg was observed whereas a significant decrease in Met, Thr, Ala, Asp, Gln, Pro, and Ser was observed (Fig. 4e). Together, these findings indicate that the Artemis I mission environment induces genotype-dependent reprogramming of amino acid utilization during early heterotrophic growth and may underlie the altered hypocotyl elongation responses observed in *ahass2* and *omr1* seedlings.

Genotype-dependent effects of the Artemis I mission on post-germination growth and rosette development

Because the essential amino acids stored in seeds are required to maintain cellular processes during the heterotrophic stage, we hypothesized that, compared with SGC WT samples, plants germinated from BCAA-enriched seeds flown in lunar orbit would have higher vigor, which would result in improved overall growth. To test this, Col-0 and the BCAA-enriched mutants were grown from seeds exposed to SGC or Flight conditions and analyzed at the rosette stage (Fig. 5a, Supplemental Data 10). We found visibly larger rosettes in Col-0 and *ipms1* seedlings derived from seeds that experienced flight conditions compared to SGC, whereas *ahass2* and *omr1* displayed similar rosette sizes between treatments (Fig. 5b). Because each flat was established with 32 healthy seedlings for every genotype and treatment, these differences were not attributed to variation in seedlings number or plant density. Quantification of rosette area confirmed a significant increase in rosette size for Col-0 and *ipms1* seedlings grown from seeds that experienced Flight compared with SGC (**P < 0.001; Fig. 5c). In contrast, no statistically

significant differences were observed between SGC and Flight for *ahass2* or *omr1*, indicating that these genotypes do not exhibit an Artemis I mission-induced enhancement of rosette growth.

Together, these results demonstrate that post-germination growth responses to the Artemis I mission environment are genotype specific, with Col-0 and *ipms1* showing enhanced rosette expansion, while *ahass2* and *omr1* remain largely unresponsive. These findings suggest that germplasm carrying distinct perturbations in BCAA-related pathways exhibited different growth responses following the Artemis I lunar mission.

Discussion

The successful deployment of plant-based systems for long-duration space exploration largely hinges on seeds' ability to maintain viability, nutritional quality, and growth potential following exposure to the space environment. Using the Artemis I mission as a platform for deep-space exposure, this study aimed to provide insights into how transit beyond LEO influences seed metabolism and subsequent plant performance, with a focus on BCAA homeostasis. We focused on BCAAs due to the essential role of these amino acids in the human diet, and due to the nature of BCAAs as key regulators of central metabolism and stress responses in plants, all of which constitute phenotypic traits that could be leveraged to optimize crop performance and food quality during long-duration space missions. Our results demonstrate that, while seed viability and early developmental processes are resilient to the Artemis I mission environment, amino acid metabolism and post-germination growth responses are conditioned by amino acid homeostasis, which can be genetically controlled.

To place these responses into context, environmental monitoring during the Artemis I mission showed that seeds experienced dynamic temperature and humidity conditions at the beginning and ending of the mission and remained stable throughout. This is an important consideration, because maintaining stable storage conditions during transit beyond LEO is critical for preserving seed integrity and for this study when interpreting the post-flight biological responses.

Because radiation characterization and impact to biological organisms was a major focus of the Artemis I lunar mission, dosimetry measurements provided an additional and important framework for interpreting the environmental conditions experienced by the seeds. Radiation measurements within the Science Bag 1, where the seeds were stored, showed that the absorbed dose of radiation was higher relative to ground control samples. Although Artemis I was a relatively short-duration mission and therefore does not model the chronic deep-space exposure expected during missions such as those to Mars, the seeds nonetheless experienced an acute ~ 25-day period of elevated radiation relative to ground controls. Based on previous plant radiation studies, however, an exposure of this magnitude, particularly within Science Bag 1 and under the shielding provided by Orion, is unlikely to have had a major biological effect on plants^{26–28}.

Furthermore, radiation exposure was consistent across sample positions. Such a uniformity in radiation dose and minimal variability in the microenvironment support the interpretation that the observed biological differences arise from intrinsic genetic and metabolic properties of the samples rather than positional artifacts. The temporal variability in temperature and humidity verified and radiation profile measurements in our study, represents a regime that seeds are likely to encounter during future transit to the Moon.

Previous studies have shown that spaceflight can broadly reprogram plant metabolism, including shifts in primary metabolic pathways and stress-associated metabolites, yet how deep-space exposure influences seed amino acid homeostasis is unknown. In our work, we found that Artemis I mission induced significant remodeling of amino acid composition in dry seeds, but only in specific genetic backgrounds. Specifically, the BCAA-enriched mutant *ipms1* exhibited pronounced increases in multiple amino acids, including essential amino acids and BCAAs, whereas WT, *ahass2*, and *omr1* seeds showed minimal changes. These findings indicate that amino acid metabolism in seeds is not uniformly sensitive to the Artemis I mission; rather, it is shaped by pre-existing metabolic states. Importantly, the observed increases in essential amino acids did not compromise seed viability or germination, suggesting that nutritional enhancement and seed resilience are not mutually exclusive after exposure to the Artemis I mission conditions.

Despite clear metabolic changes at the seed level, germination, seed viability, and early post-germination development was largely unaffected by the Artemis I mission across all genotypes. This resilience during early development suggests that seeds possess buffering mechanisms that preserve core developmental programs following the Artemis I mission, even with altered metabolic composition. In contrast, the Artemis I mission exerted strong effects on amino acid homeostasis during early seedling development, particularly in genotypes with perturbed BCAA biosynthesis. The divergent metabolic responses observed among *ahass2* and *omr1* seedlings indicate that disruption of critical nodes in BCAA biosynthesis sensitizes plants to Artemis I mission-induced metabolic reprogramming. These findings support a model in which BCAA metabolism contributes to the integration of environmental stress signals during early growth, influencing downstream metabolic balance rather than early developmental viability per se.

Notably, the Artemis I mission environment promoted enhanced rosette expansion in WT and *ipms1* plants, whereas *ahass2* and *omr1* remained largely unresponsive. This genotype-dependent growth plasticity suggests that specific alterations of BCAA metabolism can promote growth after exposure to the Artemis I mission conditions, while others constrain it. The diverging responses among BCAA-enriched mutants emphasize that elevated amino acid levels alone are insufficient to predict growth outcomes; instead, the regulatory architecture of metabolic pathways appears to be a critical determinant.

Collectively, this study demonstrates that the Artemis I mission environment does not uniformly impair plant performance but instead interacts with the plant's metabolic state to shape physiological and

growth outcomes. By identifying BCAA metabolism as a key modulator of plant responses to deep-space exposure, these findings provide actionable insights for germplasm selection and metabolic optimization of crops for future lunar and Mars missions. More broadly, this work highlights the importance of integrating metabolic traits into strategies for developing resilient, nutritionally optimized plant systems to support sustainable human exploration beyond low Earth orbit.

Methods

Plant material

ipms1-1 (At1g18500; ABRC stock CS69733), *ahass2-1* (At5g16290; ABRC stock CS69724), *omr1-11* (At3g10050; ABRC stock CS69720), and Col-0 were grown in an environment-controlled growth chamber under standard *Arabidopsis thaliana* growth conditions. Parent plants of each genotype were grown simultaneously in the same chamber. Seeds from all plants of the same genotype were pooled to generate the Flight and synchronous ground control (SGC) seed stocks. Seeds were sieve-filtered to remove extraneous plant material and stored in air-permeable envelopes prior to transfer to the Kennedy Space Center (KSC).

Experimental set up and flight on the Artemis-I lunar mission

Thirty milligrams of seeds (approximately 1,000–2,000 seeds) from each genotype were loaded into 1.7 mL tubes. Six tubes were placed inside 50 mL conical tubes containing cotton packing, sealed with parafilm, and housed within foam containers fitted into Science Bag 1. Temperature and radiation exposure within Science Bag 1 were monitored throughout the Artemis I lunar mission. An identical seed arrangement was maintained as a synchronous ground control (SGC) at the Kennedy Space Center (KSC).

Science Bag 1 was mounted to the backbone of the Orion capsule. The Artemis I mission launched from KSC on November 16, 2022, and returned to Earth on December 11, 2022, completing a 25-day mission that traveled beyond low Earth orbit into a distant retrograde orbit around the Moon. Following recovery at KSC, the biological samples were transported to Michigan State University for downstream analyses, while the radiation detectors were transported to NASA Johnson Space Center for measurements and analysis. During the mission, the flight samples were exposed to the Artemis I lunar mission environmental conditions including radiation, microgravity, and gravitational transitions during launch and re-entry.

Germination, viability and heterotrophic assays

Half-strength Linsmaier and Skoog ($\frac{1}{2}$ LS) sterile culture medium containing micronutrients and vitamins was prepared from a commercially available premixed formulation (Caisson Labs) and supplemented

with 1% sucrose and 1% agar prior to autoclaving. Fifty milliliters of medium were dispensed into each Petri plate.

Seeds from each genotype were surface sterilized prior to plating. Seeds were washed once with 70% ethanol, followed by treatment with 10% bleach containing 0.5% Tween-20, and then rinsed three times with sterile H₂O. Approximately 100 seeds per genotype were wet plated for germination and viability assays. For hypocotyl elongation assays, 20 seeds per plate were plated in a single row.

All plates were sealed with surgical tape and cold stratified to synchronize germination by wrapping in aluminum foil and incubating at 4°C in the dark. For germination and viability assays, plates were then placed vertically in a Percival growth chamber under standard *Arabidopsis* growth conditions (100 $\mu\text{E m}^{-2} \text{ s}^{-1}$ light, 16 h light/8 h dark photoperiod, 22°C). For hypocotyl elongation assays, plates remained wrapped in aluminum foil and were incubated in the same growth chamber to maintain identical temperature conditions. Seedlings were grown for five days before imaging.

Germination rate was calculated as the percentage of seeds exhibiting radicle emergence relative to the total number of seeds plated. Seed viability was calculated as the percentage of seedlings that developed expanded green cotyledons and exhibited post-embryonic root growth relative to the total number of seeds plated.

For the heterotrophic assay, hypocotyl length of each germinated seedling was measured using ImageJ software. The average hypocotyl length was calculated for each genotype.

Autotrophic assay

Thirty-two viable seedlings from each genotype identified in the germination and viability assays were transplanted into individual pots containing standard *Arabidopsis* growth soil and grown under standard conditions in a temperature-controlled growth chamber. After four weeks of growth, adult plants were imaged and shoot area was quantified for each plant using ImageJ software.

Free amino acid extraction and LC-MS/MS analysis

For dry seed free amino acid quantification, approximately 5 mg of dry seeds from three individual plants (biological replicates) of Col-0 and each mutant line were extracted and analyzed individually for free amino acid levels. For dark-grown seedling free amino acid quantification, approximately 10 mg of tissue from five individual plants (biological replicates) of Col-0 and each mutant line were extracted and analyzed individually.

Seed and dark-grown seedling amino acid extractions for LC-MS/MS (liquid chromatography–tandem mass spectrometry) analysis followed a previously described method²³ with minor modifications. The extraction buffer contained 12 isotope-labeled amino acid standards (Sigma, Cat. #767964).

Approximately 5 mg of dry seeds or 10 mg of fresh dark-grown seedlings were disrupted using a TissueLyser (Qiagen). Tissue was homogenized in 400 μL extraction buffer, incubated at 90°C for 5 min, cooled on ice, and centrifuged at 13,000 $\times g$ for 10 min at 4°C. The supernatant was transferred to 0.45

µm low-binding hydrophilic polytetrafluoroethylene filter units (Millipore) and centrifuged at $2,000 \times g$ for 30 min. The flow-through was collected and stored at -80°C until analysis.

Calibration standards were prepared containing the 12 heavy-labeled amino acid standards at the same concentrations as in the extraction buffer together with the 20 proteinogenic amino acids (Sigma-Aldrich) at concentrations ranging from 0.1 to 100 µM to generate calibration curves. Samples were analyzed by LC–MS/MS at the Michigan State University Mass Spectrometry and Metabolomics Core.

Statistics and reproducibility

All statistical analyses were performed in R 2025.09.1 + 401). For amino acid quantification experiments, biological replicates consisted of independently collected seed or seedling samples derived from separate plants. When technical replicates were present, measurements were first averaged within each biological replicate prior to statistical analysis.

For free amino acid abundance (FAA) analyses, treatment effects were assessed using analysis of variance (ANOVA). To evaluate treatment effects within each genotype, one-way ANOVA models were performed separately for each genotype, followed by post hoc comparisons using estimated marginal means (emmeans). When multiple comparisons were conducted across amino acids within a genotype, P-values were adjusted using the Benjamini–Hochberg false discovery rate correction.

For hypocotyl measurements, individual seedling lengths were averaged within each biological replicate before analysis. Treatment effects (synchronous ground control, SGC, versus flight) were then evaluated within each genotype using one-way ANOVA.

Germination and viability percentages and rosette area from Artemis I mission-exposed seeds (Flight) and synchronous ground control seeds (SGC) were compared separately for each genotype using two-tailed Welch's two-sample t-tests implemented with the `t.test` function.

Data are presented as individual biological replicates unless otherwise indicated. Horizontal bars represent median values. Statistical significance thresholds were defined as $P < 0.05$ (*), $P < 0.01$ (**), and $P < 0.001$ (***); non-significant differences are indicated as “ns”. The number of biological replicates and statistical tests used are indicated in the corresponding figure legends.

Data availability

All data generated or analyzed during this study are included in this published article and its supplementary information files.

Declarations

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Author contributions

J.T., E.A., F.B., designed the study; J.T. and R.G. performed the experiments, acquired and analyzed the data; J.T. R.G. and F.B. interpreted the data; J.O’K. developed the LC-MS/MS method; Y.Z., K.S. provided administrative services; D.D., and R.G. selected and managed radiation dosimetry; J.T. and F.B. wrote the manuscript.

Competing interests

The authors declare that they have no competing interests.

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Table

Table 1: Radiation exposure during the Artemis mission in space and post flight on Earth.

Location	Condition	RAM (mGy)	CAD (mGy)
Upper container in Science Bag 1	Flight	11.7 ± 0.4	12.1 ± 0.5
Lower container in Science Bag 1	Flight	11.4 ± 0.4	12.6 ± 0.5
Kennedy Space Center	Post flight Earth	n/a	0.48 ± 0.02

Total absorbed dose in miliGray (mGy) during synchronous ground control (SGC) and Artemis I lunar mission (Flight). The mission dose of radiation using crew active dosimeters (CAD) and radiation area monitors (RAM) positioned in the upper and lower containers of Science Bag 1. The radiation instrument measurements for Flight are reported from November 16, 2022, to December 11, 2022. For the synchronous ground control (SGC), a CAD was used post flight to monitor the radiation exposure on Earth. The total ground dose in mGy was recorded from January 01, 2023, to June 30, 2023. n/a, no available data.

Figures

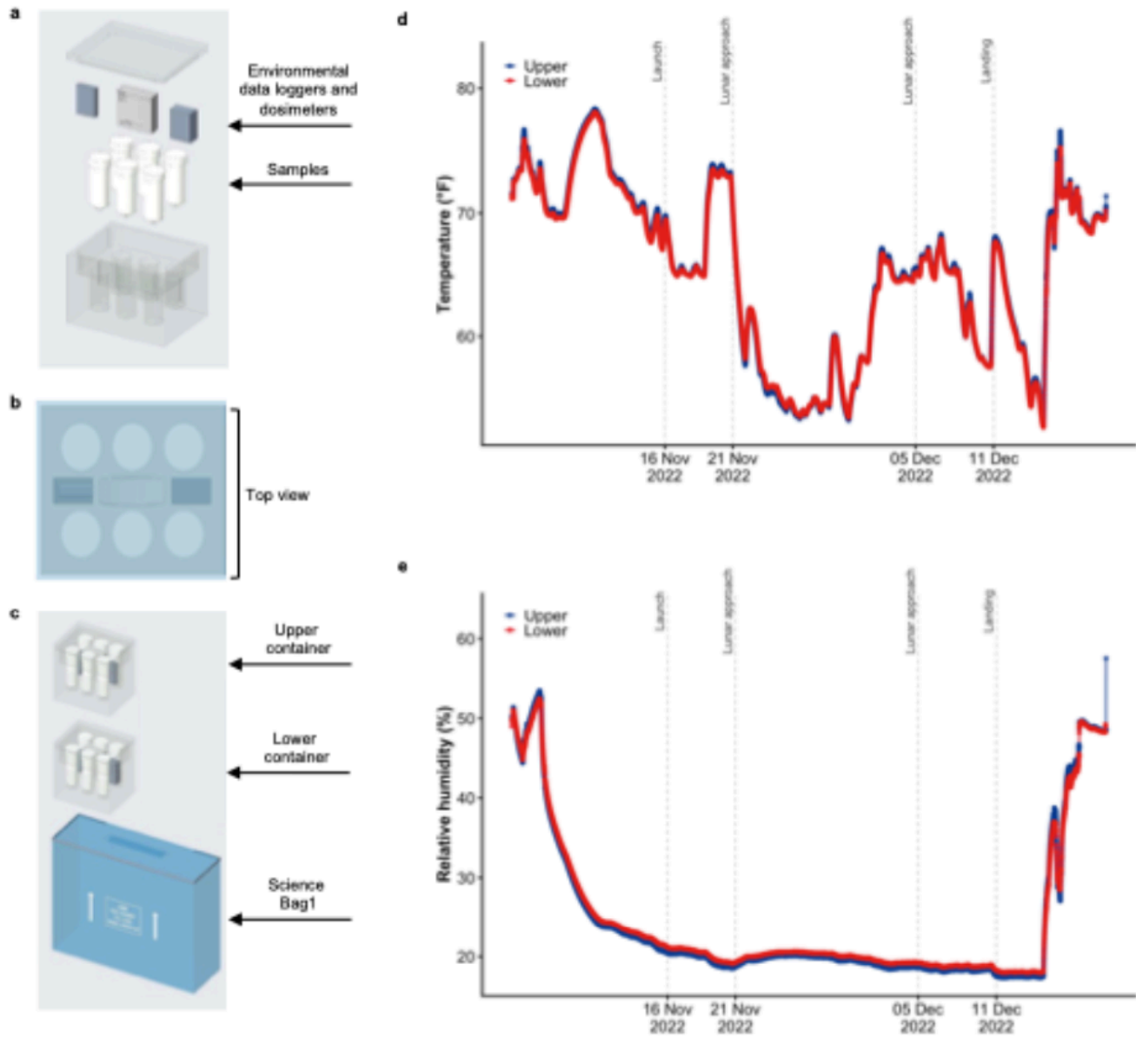


Figure 1

Environmental set up, flight timeline, and environmental conditions experienced by seed samples during the Artemis I mission. (a) A diagram depicting the position of the sample enclosure internal components including the environmental data loggers, radiation area monitor (RAM) and crew active dosimeter (CAD), and samples housed in Falcon tubes. (b) top views of the internal sample layout, illustrating the spatial arrangement of biological samples and environmental monitoring hardware within the container (c) Placement of upper and lower sample containers within Science Bag 1. The relative positions of the upper and lower seed containers are shown within the flight-certified soft stowage bag. (d) Temperature (°F) and (e) relative humidity (%) recorded by upper and lower data loggers during the mission. Vertical dashed lines indicate major mission events (launch, lunar approach, and landing).

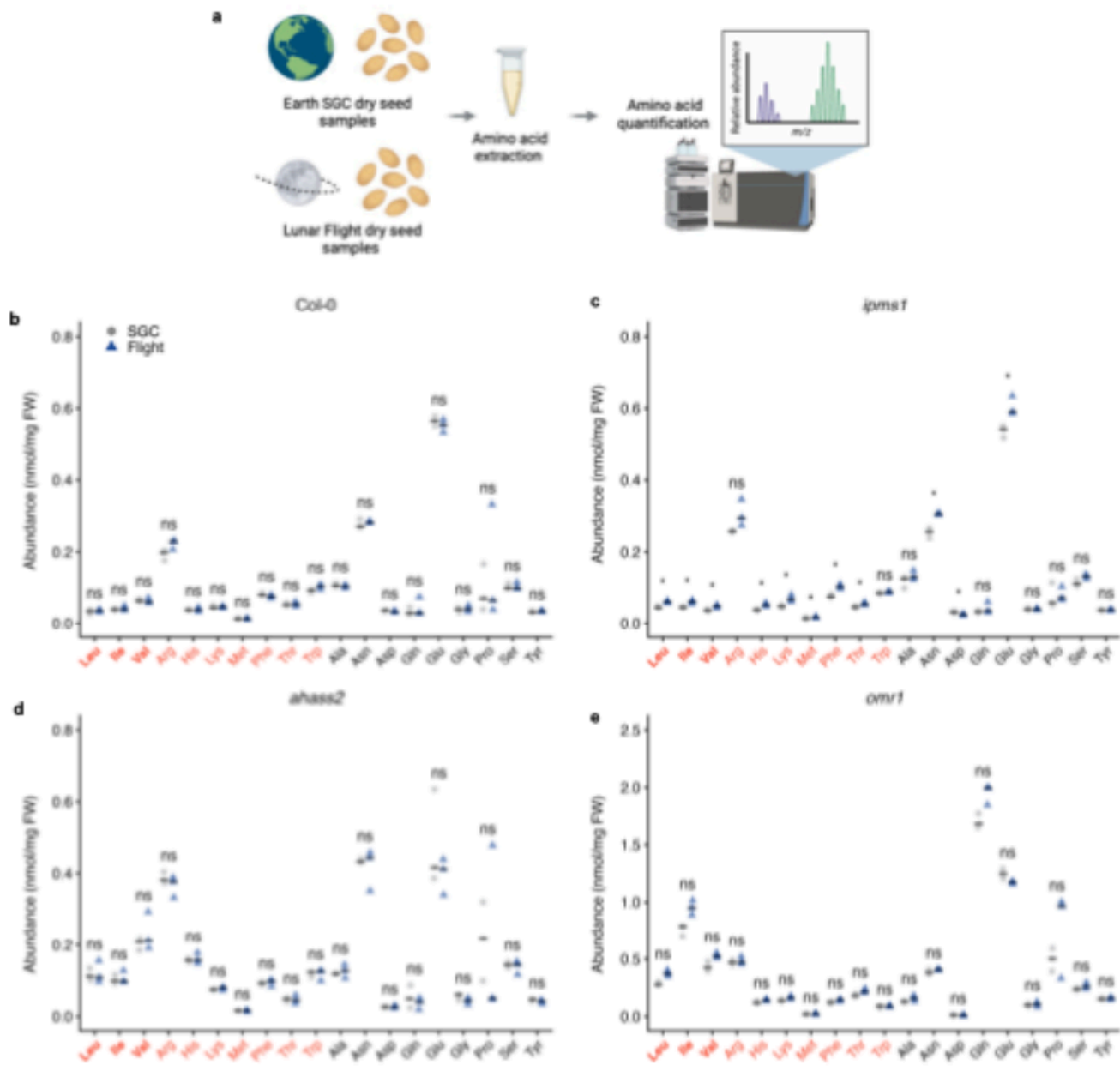


Figure 2

Genotype-dependent changes in free amino acid abundance in dry seeds following the Artemis I mission. (a) Experimental overview. Dry seeds from Earth-stored controls (SGC) and lunar Flight samples were subjected to amino acid extraction followed by quantitative analysis using liquid chromatography-mass spectrometry (LC-MS). (b-e) Free amino acid abundance (nmol mg⁻¹ FW) in dry seeds of *Col-0* (b), *ipms1* (c), *ahass2* (d), and *omr1* (e) following the Artemis I mission. Grey circles represent SGC controls and blue triangles represent Flight samples. Branched-chain amino acids (BCAAs; Ile, isoleucine; Leu, leucine; Val, valine) are indicated in bold. Essential amino acids (His, histidine; Lys, lysine; Met, methionine; Phe, phenylalanine; Thr, threonine; Ile, Leu, and Val) are highlighted in red. Other quantified amino acids include Ala (alanine), Arg (arginine), Asn (asparagine), Gln (glutamine), Glu (glutamate), Gly

(glycine), Pro (proline), Ser (serine), Trp (tryptophan), and Tyr (tyrosine). Values represent biological replicates for each genotype ($n = 3$). Statistical significance between SGC and Flight samples within each genotype was assessed independently using one-way ANOVA; $p < 0.05$ is indicated by an asterisk (*), and “ns” denotes not significant. (a) Generated using Biorender.com.

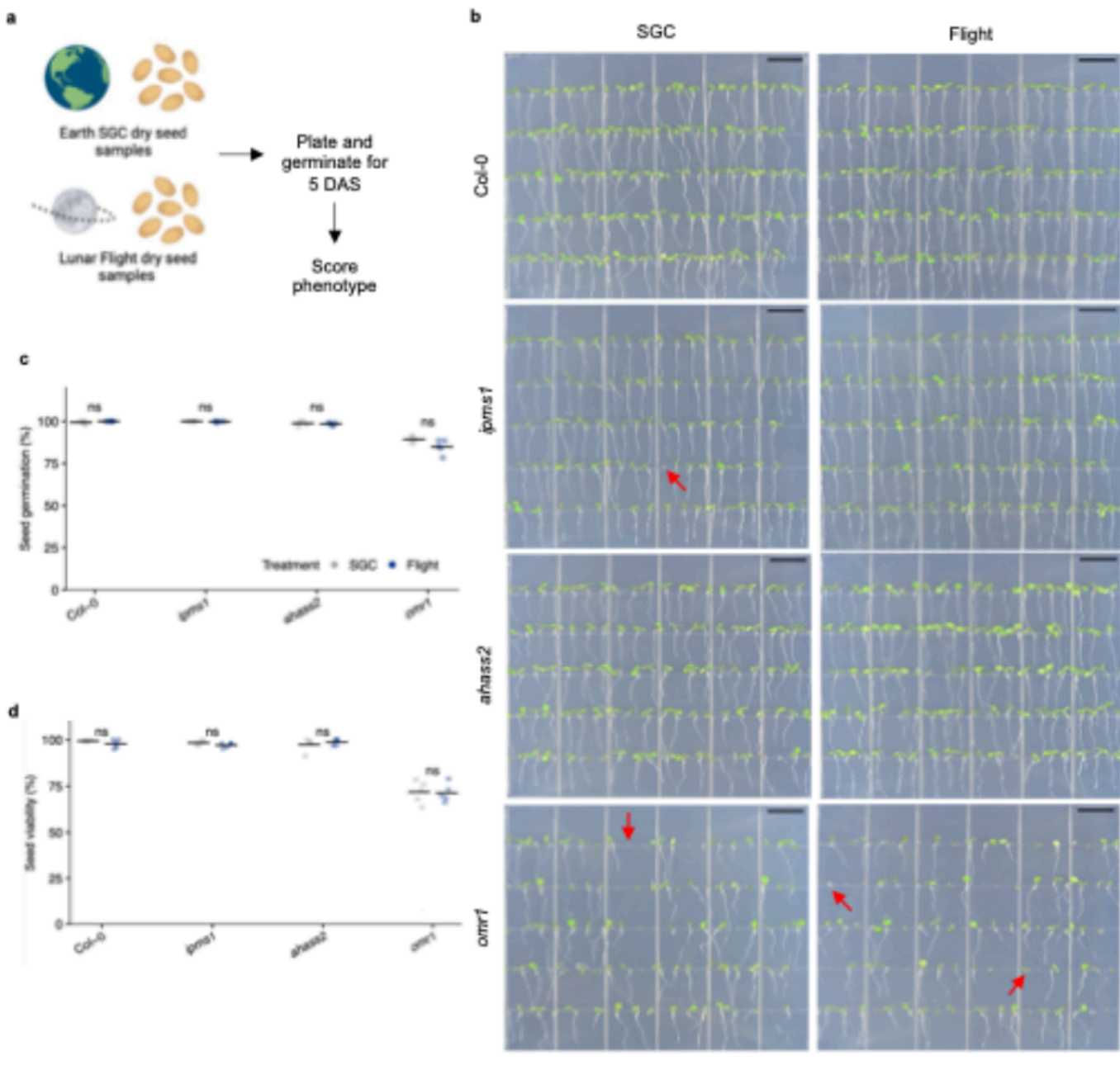


Figure 3

Seed germination and viability are maintained following the Artemis I mission. (a) Experimental overview. Dry seeds from synchronous ground controls (SGC) and lunar flight (Flight) samples were assessed for germination and early seedling establishment. (b) Representative images of 5-day-old seedlings derived from SGC and Flight seeds for Col-0, *ipms1*, *ahass2*, and *omr1*. Red arrows indicate

seeds that failed to germinate or exhibited compromised viability. Scale bars indicate 1 cm. (c) Germination and (d) viability percentage for SGC (grey circles) and Flight (blue circles) samples across genotypes. Each point represents a biological replicate ($n = 3$); horizontal bars indicate the mean. Students T-tests for statistical comparisons were performed within genotype; “ns” denotes not significant differences between SGC and Flight samples. (a) Generated using Biorender.com.

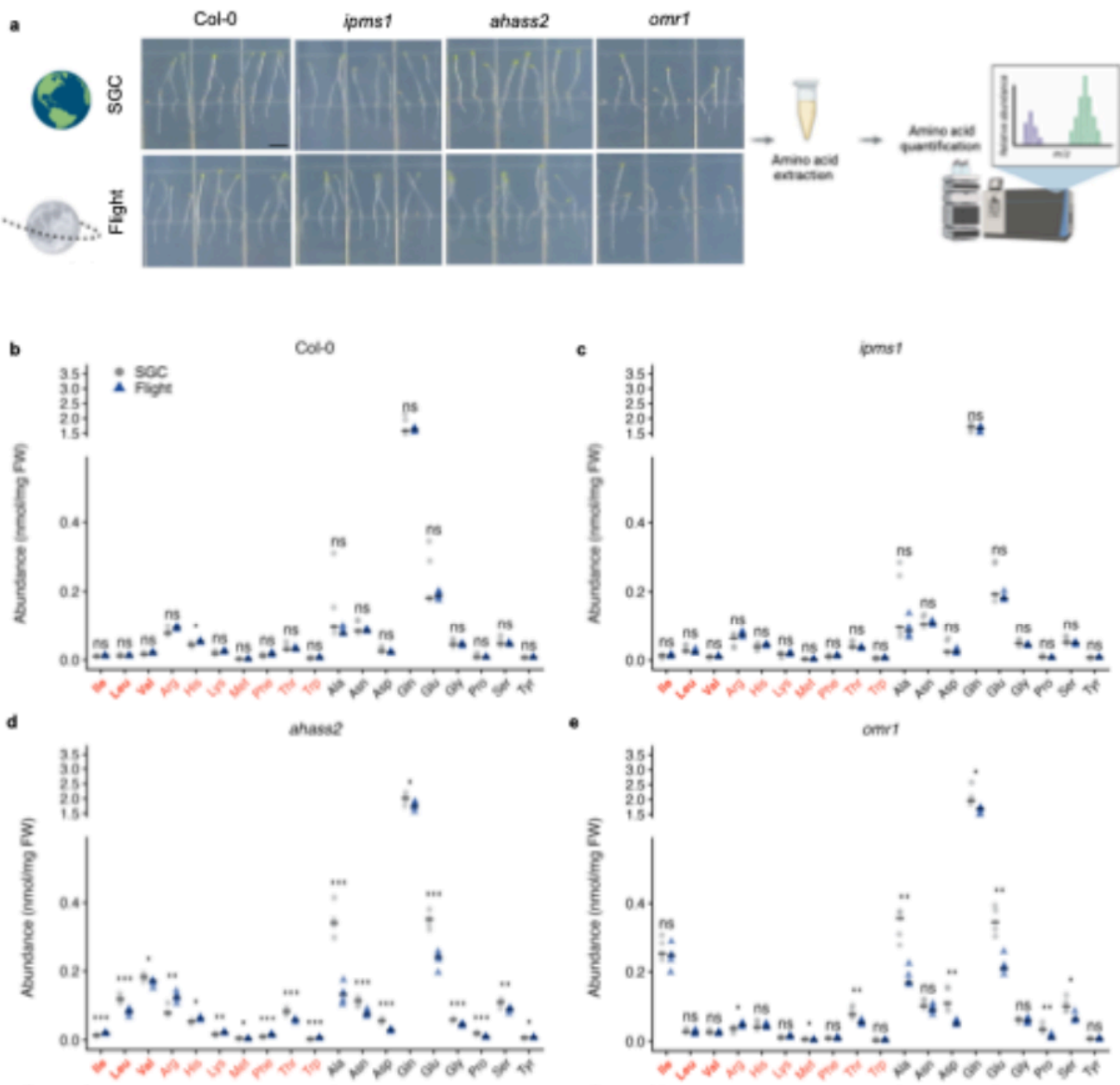


Figure 4

Free amino acid abundance of dark-grown seedlings derived from Artemis I mission-exposed seeds.

(a) Experimental overview. Dry seeds from synchronous ground controls (SGC) and Flight samples were surface sterilized, plated on standard agar medium, cold stratified to synchronize germination, and grown in the dark for 6 days. Representative images of 6-day-old dark-grown seedlings derived from SGC

(top row) and Flight (bottom row) seeds. Then amino acid extraction and quantification by LC-MS was carried out. Made using BioRender.com. Scale bars indicate 0.5 cm. (b–e) Free amino acid abundance (nmol mg⁻¹ FW) of dark-grown seedlings of Col-0 (b), *ipms1* (c), *ahass2* (d), and *omr1* (e). Grey circles represent SGC samples and blue triangles represent Flight samples. Each point corresponds to a biological replicate ($n=5$); horizontal bars indicate the mean. Branched-chain amino acids (BCAAs; Ile, isoleucine; Leu, leucine; Val, valine) are indicated in bold. Essential amino acids (His, histidine; Lys, lysine; Met, methionine; Phe, phenylalanine; Thr, threonine; Ile, Leu, and Val) are highlighted in red. Other quantified amino acids include Ala (alanine), Arg (arginine), Asn (asparagine), Gln (glutamine), Glu (glutamate), Gly (glycine), Pro (proline), Ser (serine), Trp (tryptophan), and Tyr (tyrosine). Statistical comparisons between SGC and Flight samples were performed independently within each genotype using one-way ANOVA followed by Tukey's post hoc multiple-comparison test. Asterisks denote significant differences ((*) = $p < 0.05$; (**) = $p < 0.01$; (***) = $p < 0.001$), and "ns" indicates not significant.

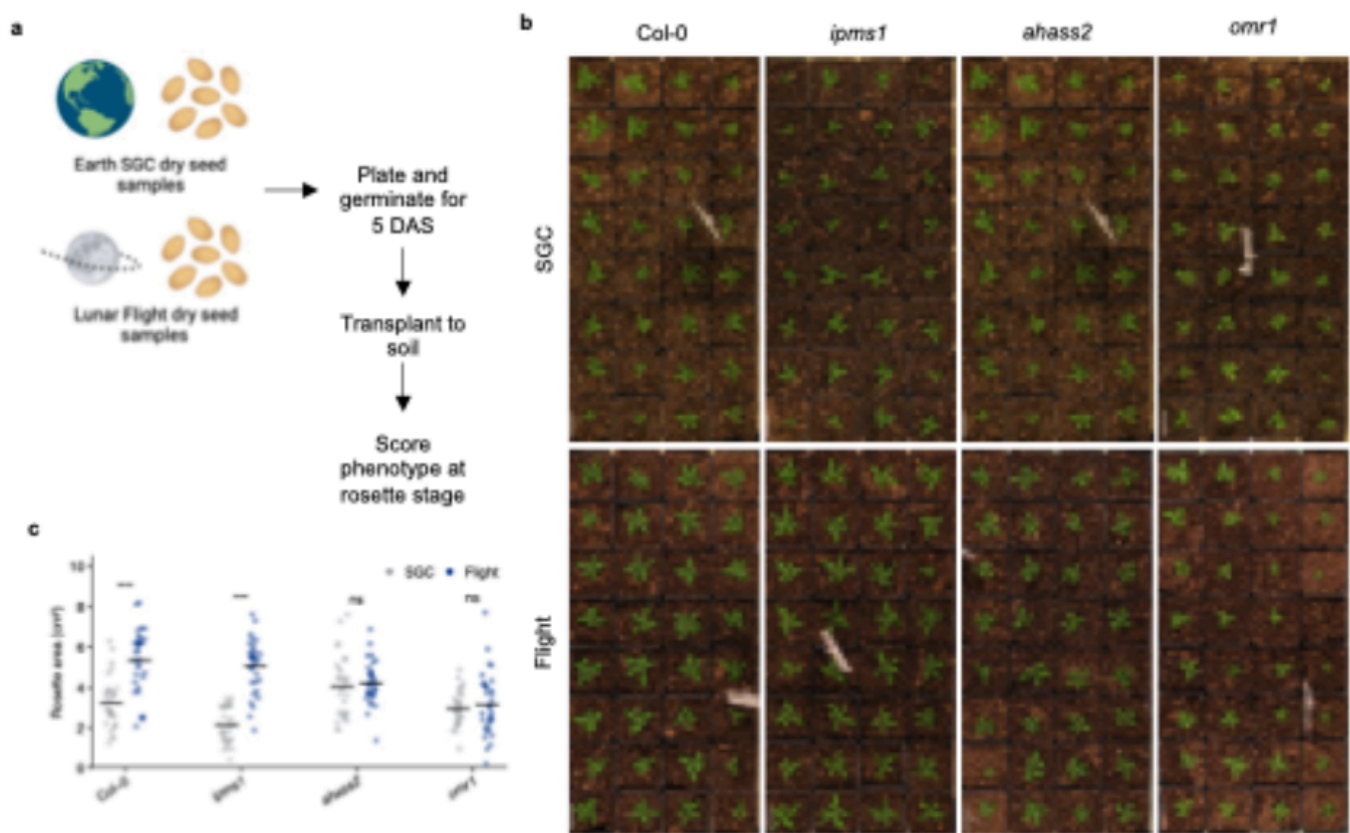


Figure 5

Rosette growth of Arabidopsis seedlings derived from seeds exposed to the Artemis I mission environment (Flight) and synchronous ground control (SGC). (a) Schematic illustrating the origin of seed samples used in the experiment: Earth SGC dry seeds and lunar Flight dry seeds. (b) Representative images of Arabidopsis thaliana seedlings grown in soil following germination. Genotypes shown include Col-0, *ipms1*, *ahass2*, and *omr1*. Scale bars indicate 1 cm. (c) Rosette area (cm²) measured for seedlings

derived from Flight and SGC seeds. Grey circles represent SGC samples and blue triangles represent Flight samples. Horizontal bars indicate the mean. Data represent mean $\pm$ SD. Asterisks indicate statistically significant differences between Flight and SGC within each genotype ((***) = $p < 0.001$); ns, not significant.

Supplementary Files

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