Gravitational and Space Research

Space Flight Cultivation for Radish (*Raphanus sativus*) in the Advanced Plant Habitat

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Abstract

*In preparation of a flight experiment, ground-based studies for optimizing the growth of radishes (*Raphanus sativus*) were conducted at the ground-based Advanced Plant Habitat (APH) unit at the Kennedy Space Center (KSC), Florida. The APH provides a large, environmentally controlled chamber that has been used to grow various plants, such as *Arabidopsis*, wheat, peppers, and now radish. In support of National Aeronautics and Space Administration (NASA)'s goals to provide astronauts with fresh vegetables and fruits in a confined space, it is important to extend the cultivation period to produce substantial biomass. We selected *Raphanus sativus* cv. Cherry Belle as test variety both for preliminary tests and flight experiments because it provides edible biomass in as few as four weeks, has desirable secondary metabolites (glucosinolates), is rich in minerals, and requires relatively little space. We report our strategies to optimize the growth substrate, watering regimen, light settings, and planting design that produces good-sized radishes, minimizes competition, and allows for easy harvesting. This information will be applicable for growth optimization of other crop plants that will be grown in the APH or other future plant growth facilities.*

Keywords

Advanced Plant Habitat • Arcillite, Red and Blue light • *Raphanus sativus* • Science Carrier

INTRODUCTION

National Aeronautics and Space Administration (NASA), European Space Agency (ESA), Japan Aerospace Exploration Agency (JAXA), and companies like SpaceX, Boeing, Blue Origin, and Sierra Nevada share the common interest of deep space exploration and establishing bases on Moon and Mars in the near future (Cichan et al., 2017; Musk, 2017; Vernikos et al., 2016). However, a major factor that interferes with human space exploration is the enormous costs of launching and resupplying resources from Earth. Therefore, developing robust technologies that enable sustainable long-duration human operations in space will be crucial in the coming years. These endeavors depend on the provision of a nutritious diet that does not rely on Earth-dependent supply chains. The initial goal is to supplement astronauts with fresh food that provides easily absorbed nutrients, vitamins, and biomass. However, the ultimate goal is becoming independent of resupplies and to reduce storage times for prepared food, which deteriorates over time (Cooper et al., 2017). This challenge faces major obstacles because plant growth facilities must function under weightlessness conditions during a Mars transit and in reduced gravitational regimes on Mars (0.38 g) or the Moon (1/6th of Earth's gravity). In addition, the necessarily closed environment must

accommodate space and substrate at a depth suitable to grow larger plants in an environment that operates independently of sunlight or Earth's gravity.

The Advanced Plant Habitat (APH), a recent addition to the International Space Station (ISS) is a plant growth chamber (GC) of ~80 L growth volume, capable of hosting long-term studies. Further, more than 180 sensors continuously monitor environmental variables (e.g., temperature, relative humidity, pressure, CO₂, light intensity, root zone moisture and temperature, water delivery, water reclamation, power consumption, and air velocity, among many others) to support whole plant testing (Monje et al., 2020). The APH consists of the GC, the Growth Light Assembly (GLA), and environmental control system (ECS), which is under the control of Plant Habitat Avionics Realtime Manager in Express Rack (PHARMER). The GC contains the science carrier (SC) that holds the substrate for the plants. The SC consists of four quadrants that use porous clay (1–2 mm arcillite) and water is delivered via four porous tubes per quadrant. Moisture content is continuously monitored. However, no direct feedback loop exists between moisture sensors and water delivery. The GLA consists of five tunable LED banks – blue (450 nm, 0–400 $\mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$), green (525 nm, 0–100 $\mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$), red (630 nm, 0–600 $\mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$), white

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(4700K, 0–600 $\mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$), and near infra-red (735 nm, 0–50 $\mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$) that are capable of producing different light spectra and fluence values.

The APH had its first test run on the space station in Spring 2018 using *Arabidopsis thaliana* and dwarf wheat as part of the PH-01 experiment under (PI: Dr. Norman Lewis). The second experiment was launched in October 2020, and two grow-outs were conducted in November and December of 2020. We grew *Raphanus sativus* cv. Cherry Belle plants from seeds for 27 days on the ISS. The plants were harvested and stored in the Minus Eighty Degree ($^{\circ}\text{C}$) Laboratory Freezer for ISS (MELFI). We expect sample-return to occur by mid-2021. The primary research objective of PH-02 research is to assess metabolic, physiological, and genetic responses of radishes grown on the ISS and identify the effects of the space environment (predominantly weightlessness and elevated CO_2) on metabolite content, flavor, mineral uptake, and overall growth. This knowledge will facilitate the transition from earth-bound cultivation of plants to growth conditions in space. Radish was selected because it not only has a short cultivation time and is also capable of accommodating genetic information from *Arabidopsis*, since it is a member of the Brassicaceae. Radish also has prominent secondary metabolites such as glucosinolates (Musgrave et al., 2005) and accumulates diverse minerals.

This paper recounts our experiences from ground-based testing in the preparation for the space experiment. We conducted two Science Verification Tests (SVT) and two Experimental Verification Tests (EVT). Each test improved our knowledge on radish responses to closed system cultivation, improved watering regimen, substrate, nutrient requirements, suitable light quality, and fluence settings. We report the effects of these changes on radish plant biomass, leaf area, and productivity based on mineral and nitrogen content. The results from these tests were essential for the flight experiment.

MATERIALS AND METHODS

Plant Material

To select the most reliable variety of *Raphanus sativus*, we tested germination rates of seeds from various cultivars (Table 1). Fast and reliable germination (24 h after imbibition) and uniform seed size were the main selection criteria. Based on the projected maturation time and reliable germination, we chose the variety “Cherry Belle” for our experiments.

Seed Sanitation

To minimize the effect of surface-borne microorganisms, we sanitized seeds based on several protocols, including bleach

Table 1. Tests of germination rate and percentage of different varieties of *Raphanus sativus* from sources (1 and 2) shown below.

	Germination		
	Fast (1 day)	Slow (3 days)	None
Organic Sparkler White top Radish ¹	23%	23%	53%
Organic Purple Plum Radish ¹	57%	0%	43%
Organic German Giant Radish ¹	63%	10%	27%
Organic Champion Radish ¹	97%	0%	3%
Cherry Belle Radish ¹	97%	0%	3%
Roxanne F1 Hybrid Round Radish ²	70%	13%	17%
Sora OG, Round Radish ²	87%	7%	7%
Rudolph OG, Round Radish ²	67%	23%	10%
Rover F1, Hybrid Round Radish ²	90%	10%	0%

¹Sustainable Seed Company.

²Johnny's Selected Seeds.

Table 2. Seed sanitation* and percentage of germination of *Raphanus sativus* var. “Cherry Belle” after 1 day and 3 days.

Treatment	1 day	3 days	None
Bleach (10 min), EtOH (5 min)	33%	0%	67%
Bleach (7 min), +24 h, EtOH (3 min)	43%	0%	57%
Bleach (7 min), +48 h, EtOH (3 min)	43%	40%	17%
Bleach (5 min)	100%	0%	0%
Bleach (7 min)	100%	0%	0%
Bleach (10 min)	93%	0%	7%
EtOH (10 s)	87%	0%	13%
EtOH (30 s)	97%	3%	0%
EtOH (1 min)	93%	7%	0%
EtOH (3 min)	90%	7%	3%
EtOH (5 min)	83%	7%	10%
Bleach, (5 min) and EtOH (1 min)	91%	8%	0%
EtOH (1 min) and Bleach (5 min)	93%	3%	3%

*Beach was used at 20% (1:5 dilution) of commercial 5.75% hypochlorite; ethanol was used at 70% (v/v).

and ethanol (Table 2), and noticed high mortality after as little as 1-minute exposure to 70% ethanol. Therefore, sanitation was based on immersion in 20% bleach for 10 min, and three times rinsing (5 min) in autoclaved (121°C , 20 min) deionized water and draining. After complete removal of the last rinse, seeds were blotted dry with Kimwipes, and air dried for >5 h. Subsequently, the sanitized seeds were stored in autoclaved polypropylene tubes.

Table 3. MS medium with* and without chloride (MS-Cl).

Components	mg/L		Ion	mM	
	MS*	MS-Cl		MS	MS-Cl
Ammonium nitrate	1650	1450	NH ₄	20.6147	18.1170
			NO ₃	39.4080	39.8741
Boric acid	6.2	6.2	B	0.1003	0.1003
Calcium chloride anhydrous	332.2	–	Ca	2.9933	2.9648
Ca(NO ₃) ₂ × 4H ₂ O	–	700	K	20.0474	20.0474
Cobalt sulfate × 7H ₂ O	–	0.028	Co	0.0001	0.0001
Cupric sulfate × 5H ₂ O	0.025	0.025	Cu	0.0001	0.0001
Na ₂ -EDTA	37.26	37.26	Na	0.2002	0.2002
Ferrous sulfate × 7H ₂ O	27.8	27.8	Fe	0.1000	0.1000
Magnesium sulfate anhydrous	180.7	180.7	Mg	1.5012	1.5012
Manganese sulfate × H ₂ O	16.9	16.9	Mn	0.1000	0.1000
Molybdic acid (NH ₄ salt) × 4H ₂ O	–	1.25	Mo	0.0010	0.0010
Potassium iodide	0.83	0.83	I	0.0050	0.0050
Potassium nitrate	1900	1900	P	1.2491	1.2491
Potassium phosphate monobasic	170	170	Zn	0.0299	0.0299
Zinc sulfate × 7H ₂ O	8.6	8.6	SO ₄	1.7312	1.7313
			Cl ⁻	2.9934	0.0000
Grams of salts to prepare 1 L	4.3	4.5			

MS, Murashige-Skoog.

*Values are based on Sigma product M5524.

Substrate

Prior KSC-based experiments used arcillite (calcined Montmorillonite commercially available as Turface Pro League). Its porous structure, neutral pH, low density (~0.63 g · cm⁻³), and hydrophilicity suggested its use as growth substrate (Adams et al., 2014). The commercial material was sifted to obtain grains between 1 mm and 2 mm. Preliminary studies showed that arcillite does not contain necessary nutrients to support plant growth. Therefore, arcillite was supplemented with half-strength, modified (chloride-free, because of corrosion concerns) Murashige and Skoog medium (Table 3). Equal weights of dried and autoclaved arcillite and modified Murashige-Skoog (MS) medium at final concentration were combined, soaked for 24 h and dried (70°C for 72 h). The fertilized, dried arcillite can be stored indefinitely.

SC Packing and Seeding

Each of the four quadrants of the SC was filled with ~1600 mL of fertilized, dry arcillite. The substrate was filled and tamped down to fill all the available spaces in and around the sensors and porous tubes. Medical gauze was placed above the arcillite to keep the substrate in place. A hole (~5 cm diameter)

was cut in the gauze to accommodate OASIS foam (Smithers-Oasis, Kent, OH) pucks that accommodated the seeds. Another layer of medical gauze was placed on top to secure the oasis foam (Figure 1). The top-layer gauze was split by two perpendicular cuts of ~2 cm length each. In addition to retaining the foam, the gauze also provided visual feedback of the wetness and thus the water level of the entire setup. A layer of orthopedic foam with cutouts for the floral foam secured everything under the SC covers. Dry sanitized seeds were glued with water soluble glue (polyvinyl acetate, Elmer's clear glue) ~5 mm deep into the foam such that the micropyle was positioned toward the arcillite. The foam provided water for seed imbibition and germination, and its flexibility allowed for the expansion of the developing bulb (used here instead of the anatomically correct description of "swollen hypocotyl," Figure 2).

Growth Conditions

The ECS of the APH unit was set to the following parameters: Photoperiod: 16 h light/8 h dark; Temperature: 24°C day/20°C night; Relative humidity 65%; CO₂ Concentration: 3500±3% ppm; and Air speed: 0.9 m/s based on fan rotational velocity.

Figure 1. Cross sectional view of a packed SC. Not shown are porous tubes and moisture sensors embedded in the arcillite. The orthopedic foam was used to secure (slightly compress) the arcillite to prevent shifting during manipulations and eventual launch vibrations. SC, science carrier.

Figure 2. The SC quadrants with two five and nine planting positions (A). The individual planting positions show gauze covering OASIS (floral) foam. Darkening of the gauze is useful for assessing proper water dispensation into each quadrant (B). SC, science carrier.

Illumination

One of the parameters that strongly affects the development and growth of radishes is light quality and fluence (Samuoliénė et al., 2011; Yorio et al., 2001). Since the GLA is APH-specific, we used an alternate, programmable, high intensity LED based light fixtures (Heliospectra RX30), which provides up to $1000 \mu\text{mol m}^{-2} \cdot \text{s}^{-1}$ photosynthetically active radiation (PAR). These light fixtures provide a more versatile light spectrum and higher light output than the APH system but are of smaller size. The relatively lower fluence rates of the GLA were

adjusted by reducing the output of the LEDs and increasing the distance between the light fixture and the growth surface. Laboratory experiments produced comparable results to the experimental units [APH and the lower-fidelity Engineering Development Unit (EDU)] at the KSC.

Elemental analyses

Dried and ground leaf and bulb tissues (~100 mg) were digested in 2 mL aliquots of 70% trace metal grade nitric acid (Fisher Chemical A509-P212) for 72 h. The digested samples

Figure 3. ICP-OES measurements of raw arcillite (no added fertilizer) after extraction with HCL (pH 3) and with 10 mM Tris/HCL buffer (pH 6). Large quantities are released under acid conditions but absent in at pH 6. Soluble ions were less than 1/10 of the acid extract.

were diluted with nanopure water (>10 MΩ) to 10 mL, filtered, and analyzed via Inductively Coupled Optical Emission Spectrometry (Perkin Elmer, Optima 5300 DV). A multielement standard (Inorganic Ventures, Christiansburg, VA) was diluted to the same matrix concentration and used for calibration.

Nitrogen analysis

The total nitrogen (N) content from dried and ground leaf and bulb (~100 mg) tissue material was analyzed via LECO TruMac Nitrogen analyzer using EDTA as calibration material.

RESULTS

Optimization of Growth Conditions

1. Arcillite effects

Arcillite is a ceramic aggregate that can be utilized as a component of soilless media (Adams et al., 2014). We tested the capacity of plain arcillite (not supplemented with external nutrition) to support radish growth by sowing the seeds directly into the arcillite. Although the seeds germinated and the cotyledons emerged after 72 h, the seedlings did not grow further and even after 28 days post sowing, the plants remained in the seedling stage (data not shown). This observation demonstrated that arcillite does not provide necessary minerals and is not capable of supporting

long-term plant growth. The mineral content of arcillite was assessed by extractions in acidic and neutral buffers. Acid extraction (pH 3) released minerals needed for plant growth such as potassium, calcium, and magnesium in relatively low quantities but showed high values of aluminum. In contrast, neutral extractions (pH 6) resulted in much lower quantities of all ions, especially aluminum (Figure 3).

2. Fertilization

The arcillite analyses implied that plant growth required added nutrients. Previous space experiments used the slow-release fertilizer Nutricote (Massa et al., 2013). However, its prill size resulted in uneven distribution and the slow-release rate (80% in 80 days) also required a higher fertilizer load; 9.5 g vs. 2.3 g of MS salts. These constraints were the rationale to provide readily absorbable, uniformly distributed, and lower quantities of fertilizer as modified MS medium. In addition to producing sizeable radish bulbs, we were able to determine the amount of nutrients absorbed by the plants. The fertilizer load was sufficient for two growth cycles (Figure 4). Thus, the added minerals provided adequate nourishment for at least one grow-out.

3. Design of SC lids

During the initial stages of growth experiments and based on previously established protocols, we utilized SC covers with long slits and capillary matting (Cap-Mat, Figure 5A).

Figure 4. Mineral availability of arcillite infused with MS medium (un-used) and after the first and second growth cycles of radishes. MS, Murashige-Skoog.

Figure 5. Radish seedlings grown on the SC quadrant with lid containing slits and lined with "Capillary Matting Cap-Mat" (A). The Cap-Mat resulted in restricted radish bulb development (B). SC, science carrier.

This configuration was ideal for growing *Arabidopsis* and wheat (NASA 2018). However, the combination of the narrow opening and Cap-Mat restricted the expansion of the radishes and resulted in extensively deformed bulbs (Figure 5A, B). Therefore, the covers were re-designed to contain circular openings (~5 cm in diameter) that provided sufficient space for the expanding bulbs. These tests also resulted in the replacement of Cap-Mat with OASIS foam.

4. Space optimization

The newly configured quadrant lid solved the issue of misshapen radish bulbs, but the sustainable number of plants per quadrant still needed to be determined. Based on a lid design with nine and five positions, subsequent tests showed that nine radishes per quadrant resulted in overcrowding and reduced biomass compared with five positions per quadrant. The biomass per quadrant was similar regardless of the number of plants per quadrant (5 vs. 9). However, the productivity per plant was significantly higher when five, rather than nine, plants were grown per quadrant. ($P < 0.001$, Table 4). Similarly, the mineral concentration (K, Fe, Na and P) was higher in leaves of SC with five plants (Table 5). The concentration of the remainder of minerals, especially in bulb tissue, was similar irrespective of the number of plants per quadrant (Table 5). These results indicate that mutual shading had negative effects on bulb development and overall biomass (Table 4). Based on these results, we decided to grow five plants per quadrant for all the subsequent studies.

5. Watering schedule

Since water content is critical for the formation of radishes, and the water capacity is limited for granular substrate,

Table 4. Biomass and leaf area per SC quadrant containing five or nine radish plants (averages $\pm$ SE).

	Total Mass, g	Radish, g	Leaf, g	Leaf area, cm ²
N = 5	23.4 $\pm$ 4.6	12.5 $\pm$ 2.3	13.9 $\pm$ 1.5	271.3 $\pm$ 28.3
N = 9	12.5 $\pm$ 3.1	5.8 $\pm$ 2.0	7.4 $\pm$ 1.3	135.9 $\pm$ 22.0

SC, science carrier.

Table 5. Mineral content of radish leaves and storage tissue (bulbs) in mg/g fresh weight $\pm$ SD. The numbers in brackets indicate the number of plants per SC quadrant for the respective tissue.

	Al	K	Mg	Ca	Fe	Na	Si	P
Bulb [5]	1.07 $\pm$ 0.01	295.6 $\pm$ 8.7	23.4 $\pm$ 0.3	18.8 $\pm$ 0.2	0.66 $\pm$ 0.07	17.0 $\pm$ 0.6	1.02 $\pm$ 0.04	15.6 $\pm$ 0.4
Leaves [5]	1.05 $\pm$ 0.01	460.4 $\pm$ 12	169.5 $\pm$ 4.6	149.6 $\pm$ 3.3	0.94 $\pm$ 0.02	21.9 $\pm$ 0.6	2.38 $\pm$ 0.05	23.2 $\pm$ 0.5
Bulb [9]	0.97 $\pm$ 0.01	295.4 $\pm$ 7.3	30.3 $\pm$ 0.4	18.9 $\pm$ 0.3	0.32 $\pm$ 0.03	13.6 $\pm$ 0.4	0.73 $\pm$ 0.03	11.7 $\pm$ 0.3
Leaves [9]	1.04 $\pm$ 0.01	336.7 $\pm$ 7.8	182.2 $\pm$ 4.2	168.4 $\pm$ 3.7	0.65 $\pm$ 0.02	18.8 $\pm$ 0.5	2.04 $\pm$ 0.05	17.8 $\pm$ 0.4

SC, science carrier.

we measured water consumption during the cultivation of radishes gravimetrically. The averages from five 4-week long experiments showed that water consumption was largely a function of leaf area, light intensity, and temperature. The water loss increased with age from an initial range of 80 to 100 mL per day but increased to 500–600 mL per quadrant as the plants matured (Figure 6). The low water capacity of arcillite required continuous watering, especially in the last 2 weeks of the growth period. However, after the initial flood-filling, no water was added during the first 7 days (see continuous drop in moisture during that time, Figure 7) and the seeds were allowed to germinate under low wind speed (0.6 m/s) and high relative humidity (>70%). After the cotyledons emerged, the moisture levels reduced at a greater rate as the plant developed foliage (Figure 7). The moisture values of the arcillite substrate were targeted at 65% for the lower sensors and 50% for the upper sensors. The difference between lower and upper sensors is attributable to gravity effects.

6. Light conditions

Inconsistencies in the development of the bulbs between different tests prompted a detailed analysis of light settings. Laboratory studies and SVT were conducted under red light enriched illumination, which resulted in bolting and flower development (data not shown), reduced biomass of the bulbs, and large leaf area (Table 4). Since literature data suggested that blue light affects leaf area and bulb development (Samuolienė et al., 2011; Tezuka et al., 1994), subsequent tests, including the EVT, were based on increased fluence of blue light and decreased red light (Table 6). The light composition used in the EVT 2 resulted in overall increased biomass (Table 6) and reduced canopy size. This light composition was implemented for flight experiments.

Overall performance of the plants was estimated by measuring mineral and nitrogen contents from leaves and bulbs (Figure 8). Regardless of applying different light settings between SVT and EVT (Table 5), the nutritional value of the radishes based on mineral composition and nitrogen content did not change. Our data indicate that radishes are rich in calcium, potassium, magnesium, and sulfur. The higher quantities of

Figure 6. The bi-phasic water consumption of radishes cultivated in arcillite. The average of three representative experiments with five plants each growing in the same volume as a SC quadrant (dashed line). The water loss experienced by the plants and substrate remains constant during the first 10 days (red line); then plant-based enhanced transpiration increases (blue line). The dotted line approximates water consumption as a binomial function. SC, science carrier.

Table 6. Lighting schedules used in different ground control tests and the resultant radish biomass*.

Experiment	White [4100 K]	Blue [455 nm]	Green [530 nm]	Red [627 nm]	Far Red [735 nm]	Avg. Radish mass, g
SVT (5/16/19–6/13/19)	490	0	70	220	0	9.1
ΔSVT (10/3/19–10/30/19)	490	0	70	220	0	9.8
EVT (11/20/19–12/16/19)	460	30	50	220	20	4.4
ΔEVT (5/26/20–6/22/20)	335	310	60	20	0	13.9

Light values are shown as $\mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$.

EVT, experimental verification test; SVT, science verification tests.

minerals in leaves indicate that leaves are more nutritious than bulbs (Figure 8A). Similarly, nitrogen content of leaves is about twice as high as that of the bulbs (Figure 8B), which also indicates that leaves are more nutritious than the radish bulbs. These data suggest that the entire plant could be consumed.

DISCUSSION

The seemingly trivial project of cultivating well-known and characterized plants turned into a rather complex task when normal growth conditions are modified. Space cultivation is space-limited, uses porous substrate, and relies on artificial light, water supply, and air movement. All these factors constitute a complicated network of interactions that cannot be solved by optimization of individual parameters.

Specialized hardware (SC, illumination, watering system) not only requires integration and adaptation to changing requirements over the growth cycle but also differs between ground controls and space conditions. Therefore, this report compares plant performance between ground controls of increasing fidelity to space experiments. Based on our experience, optimization of growth conditions for the APH and future plant growth facilities will likely continue to require individual tests, especially if more than a single crop is to be cultivated. Remarkably, the “biology”, i.e., the seeds and their germination, proved to be one of the most reliable factors in our study. Seed selection (Table 1) and sanitation (Table 2) resulted in dependable performance throughout the three-year preparation time. Germination rate of the refrigerated (4°C) seeds remained >95%. Radish growth was mostly affected by substrate, planting density, watering, light, and environmental conditions. The strong deleterious effect of

Figure 7. Moisture readings from the EVT, (A) and a second EVT (B). The tracings show higher moisture readings for the lower sensors in all four quadrants. The more uniform tracings in (B) indicate better hardware performance than in (A). However, greater water demand toward the end of the culture time shows larger fluctuations, especially in (A). A strong drop in moisture readings especially for the lower sensors indicated plant water stress. The legend applies to A and B and describes the measurements of the moisture sensors in the SC quadrants 1 to 4 (Q) of the upper (up) and lower (low) sensor, respectively. EVT, experimental verification test; SC, science carrier.

Figure 8. Mineral (A) and nitrogen contents (B) from radish leaves and bulbs. Importantly, the higher mineral and nitrogen content of leaves than bulbs suggest that leaves be used as food source. The average nitrogen content (dashed lines in B) is about twice as high in leaves than in bulbs.

ethanol for seed sanitation suggests relying on bleach or other oxidative chemicals, rather than ethanol.

Growth Conditions and SC Design

Arcillite is one of the favored rooting systems for space because it reduces the chances of root zone hypoxia (Porterfield et al., 2000). However, it does not release sufficient minerals, and therefore cannot support long-term plant growth (Figure 3). Our approach of infusing arcillite with MS medium not only produced sizeable radishes but also allowed quantitation of the amount of nutrients utilized by the plants (Figure 4). Such information from space grown plant materials will be extremely valuable in understanding the mineral uptake of plants past the seedling stage under space environment.

The susceptibility of radish growth to plant density and spacing clearly showed improved plant performance when the number of plants per quadrant was reduced to five plants (Table 4). Although these data support the view that improved plant performance can be obtained as a result of substrate conditions and reduced shading, they do not address any below-surface competition. The APH design prevents variation and thus optimization of the root space, a notion especially important for plants that develop root or hypocotyl-based storage tissue. In radish, high planting densities are likely to affect photomorphogenic mechanisms as total light fluence and red to far-red ratios are known to reduce vegetative growth (i.e., bulb formation) and accelerate flowering (Weston, 1982). Thus, by decreasing the number of plants, at least two factors were affected; (1) relatively large-sized bulbs were obtained, and (2) the tendency of bolting was reduced. Equally important was the provision of larger growth areas rather than elongated slots as the modification reduced restrictions and injuries (Figure 5). Enhanced mechanical

stress as well as irregular expansion due to inconsistent watering resulted in defects on the bulb surface. Abrasion and reduced integrity of the bulb epidermis increases the chances of microbial growth. The overall roughness of arcillite may also contribute to an indirect, but testable, increase in potential microbial contamination. Such effects were reported in growth experiments performed with the VEGGIE hardware (Khodadad et al., 2020) and also reported in Daikon radishes (Shiina et al., 2013). High humidity but absence of precipitation in space may contribute to higher microbial loads than on earth. Thus, microbial contamination needs to be examined, especially for below-ground tissue.

Watering Regimen

The water loss data (Figure 6) demonstrates two stages of evapotranspiration. Stage 1 (day 1–12) water loss during germination and seedling establishment is minimal and essentially consists of evaporation of water from the SC surface. During stage 2 (day 13–28), the water consumption increases exponentially. Both stages are approximations, as the evapotranspiration rates are known to be affected by humidity and temperature (Nonnecke et al., 1971), plant size (Kim et al., 2011), and light quality (Lim and Kim, 2021). Under constant temperature and humidity levels in our tests, the major driving factor for water usage was leaf size and plant density (Table 4). The watering schedule in the APH was based on the age of the plants such that initial flood filling of the SC provided sufficient water for the first week. The flooding also resulted in reduced activity of the watering system and prevented drought stress during the early stages of plant growth. Additional adjustments involved the air velocity. During the flood-filled stage, the air speed was adjusted to $0.6 \text{ m} \cdot \text{s}^{-1}$ but increased to $0.9 \text{ m} \cdot \text{s}^{-1}$ during the remainder of the cultivation time.

Light Settings

The sensitivity of radishes to the spectral composition of light shows a prominent role of red and blue light. Increased blue light fluence increased the biomass of radishes (Table 6) and reduced the leaf area, which also resulted in a smaller overall canopy. A similar effect of blue light was described in several studies. Tezuka et al. (1994) reported that radish growth was promoted by UV-A light ($\lambda_{\text{max}} = 360 \text{ nm}$) and resulted in higher chlorophyll and vitamin content and larger radish formation. A study on the effect of red and blue light illumination examined combinations of red LEDs (638 nm) supplemented by 455 nm (blue) light on radish physiological indices and it showed that radishes grown under red light alone were elongated, with poor hypocotyl formation (Samuolienė et al., 2011). Interestingly, a small supplement of blue light (5%) enhanced leaf area formation. Higher proportions of blue light (10%) enhanced non-structural carbohydrate distribution between storage organs and leaves and resulted in enhanced radish formation. Thus, supplementation of the blue light not only promotes growth and development in radishes but also plays an important role in nutrient allocation. Light is a dominant stimulus for plants in weightlessness and affects phototropic and gravitropic responses (Sindelar et al., 2014; Vandenbrink et al., 2016). However, to date, the effects of light on biomass production under weightlessness conditions have not been investigated. The light effect also underscores the need to study growth beyond the seedling stage, which has been the focus of most space experiments. During early seedling development, effects of light on biomass accumulation, growth habit, and photomorphogenesis in general cannot be assessed, but these factors are important for adequate biomass production and nutritional value. Future space experiments should therefore include experiments that test the effect of light fluence, spectral composition, and (extended) photoperiod to optimize plant cultivation under space conditions. Similarly, effects of elevated CO_2 concentrations under weightlessness on mineral, vitamin, and lipid content are needed to optimize nutrition of spacefarers.

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