

Novel Microgreen Crop Testing for Space

LaShelle E. Spencer¹, Jennifer L. Gooden², Aaron B. Curry³
Amentum Services, Kennedy Space Center, FL, 32899, USA

Matthew W. Romeyn⁴, Raymond M. Wheeler⁵
NASA, Kennedy Space Center, FL, 32899, USA

and

Takiyah A. Sirmons⁶
Leidos Technologies, Johnson Space Center, TX 77058, USA

Long-duration missions beyond low-Earth orbit will encounter challenges in maintaining adequate nutrition and crew acceptability in the food system. *In situ* production of fresh produce can supplement nutrient deficiencies in the prepackaged diet. Currently, there are a relatively small number of crops that can be reliably grown in space for space crop production efforts. An intriguing area of new investigation involves novel types of microgreens that have the potential to be sources of calories, fat, carbohydrates, and protein. These sources of nutrition are not obtainable in significant quantities with current pick and eat crops. Many microgreen cultivars are also sources of nutrients of interest, such as Vitamins B1, C, and K, and elements such as potassium. Microgreens should be selected to address specific nutritional deficits, as identified by NASA's Human Research Program, with an emphasis on having a diversity of crops to meet nutritional requirements and crew acceptability. To achieve this, the concept of Crop Readiness Level (CRL) has been developed to gauge readiness of crops for spaceflight applications. CRL includes assessing environmental compatibility, food safety considerations, relevant nutritional analysis, and sensory analysis. Recent testing at Kennedy Space Center has focused on advancing the CRL of a variety of novel microgreens. These varieties were grown under $150 \mu\text{mol m}^{-2} \text{s}^{-1}$ PPFD from LED lights, 3000 ppm CO_2 , and 23°C to simulate an ISS environment. Crops were harvested and yield was assessed. Then, baseline microbiological and nutritional analysis (Vitamins B1, C, K; mineral analysis; proximate analysis) and sensory evaluation were performed. These baseline data are essential to selecting candidate crops for future missions and assessing crop production hardware and changes in environmental conditions on future crop performance and nutritional quality.

Nomenclature

<i>LED</i>	=	Light Emitting Diode
<i>CRL</i>	=	Crop Readiness Level
<i>PPFD</i>	=	Photosynthetic Photon Flux Density
<i>ISS</i>	=	International Space Station
<i>EC</i>	=	Electrical Conductivity
<i>DAP</i>	=	Days after planting
<i>APH</i>	=	Advanced Plant Habitat
<i>RGB</i>	=	Red Green Blue

¹ Horticultural Scientist, Amentum, Mail Code: LASSO-0008.

² R&D Scientist, Amentum, Mail Code: LASSO-0008.

³ R&D Scientist, Amentum, Mail Code: LASSO-0008.

⁴ Project Scientist, NASA, Exploration Research and Technology, Mail Code: UB-A.

⁵ Plant Physiologist, NASA, Exploration Research and Technology, Mail Code: UB-A.

⁶ Food Scientist, Leidos Technologies, Johnson Space Center, TX.

I. Introduction

As we move toward long-duration, exploration class missions, the capability to grow nutritious, palatable food for crew consumption during spaceflight becomes increasingly important. Critical nutrients have been proven to degrade over time in pre-packaged, thermo-stabilized meals, to the extent that they will not meet the shelf-life requirements of long-duration mission scenarios¹⁻². Supplementing these nutrients through pharmaceutical methods has not been recommended due to concerns with possible side effects, faster degradation of nutrients in supplement form, crew compliance, and lack of synergistic benefits associated with the natural combination of phytochemicals in whole-food delivery. Implementation of a pick-and-eat bioregenerative produce system in spaceflight has tremendous potential to supplement nutrition over time, while supporting crew psychosocial health through the introduction of fresh foods that increase the variety, texture, flavor, and color attributes of the food system³⁻⁴. To date, the testing of edible crops for space flight has been limited largely to leafy green species. A common approach to these studies is to simulate the types of environments that might occur in International Space Station (ISS) plant chambers like Veggie, for example, elevated CO₂, LED lighting, and typical ISS cabin temperatures and humidity. Regardless of the plant growth chambers used for ISS and future vegetable production systems, growth volume and shoot/canopy height will be limited. For example, shoot height available for the Veggie and Advanced Plant Habitat (APH) growth chamber systems onboard the ISS are 47 cm and 45 cm, respectively⁵.

A next step for supplementing food crops for space might be the inclusion of small, quick-growing plants such as microgreens. Microgreens are edible seedlings grown in a lawn and harvested after the cotyledons emerge (or soon after true leaves emerge), generally 7-14 days after planting. Most varieties of microgreens are approximately 2.5-7.5 cm tall. They come in a variety of colors, aromatic flavors, and concentrated nutritional profiles. They have historically been used in the restaurant industry to embellish food, and are commonly consumed in sandwiches, soups, and salads⁶. Recently, microgreens have gained increasing popularity as food ingredients because of their high nutrient content. Although the exact content can vary, most types tend to be rich in iron, potassium, and magnesium⁷⁻⁹. Sun et al (2013) reported that, when comparing microgreens to more mature greens, the nutrient levels in microgreens can be up to nine times higher than those found in mature greens¹⁰. Microgreens are ideally suited for cultivation in indoor environments due to their low light and water requirements¹¹. For this reason, they are being evaluated for biological performance and suitability under ISS-like environmental conditions, as well as being tested for nutritional content and acceptability.

For these studies, we wanted to 1) assess the morphology, yield, and viability of crops grown in mission relevant environmental conditions, 2) evaluate organoleptic acceptability, and 3) determine nutritional value of several microgreen cultivars. These findings can help us advance these new species and cultivars through a Crop Readiness Level (CRL) evaluation for eventual spaceflight applications.¹²

II. Materials and Methods

A. Microgreen Cultivation System

A custom, two-tiered microgreen cultivation system was utilized (**Figure 1**). Each tray (17 x 28 inches) contained three troughs (5.52 x 28 inches). Trays were fed via a custom 40 L nutrient solution tank constructed of black polycarbonate. Each tray was provided solution, which was recirculated utilizing external pumps (Little Giant, 3-MDX PN2P579). Pumps were controlled via OPTO22 software customized to provide up to eight irrigation events with user-selected times and durations.

B. Seeds

A complete list of all cultivars screened can be found in **Table 5**. Seeds were sourced from commercially well-known vendors such as [Johnny's Selected Seeds](#) (Winslow, ME) and [True Leaf Market/Kitazawa Seed](#) (Salt Lake City, UT). For these tests, seeds labeled "for microgreens" were sought after first, with standard seed types selected in the absence of microgreen seed.

Prior to planting, the weight of the seed needed to plant a 4 x 4 in area was weighed out and broadcast within that area.



Figure 1. Microgreen cultivation system installed in reach-in controlled environmental growth chamber. Here, lower tray is removed to view nutrient solution tank and pump.

C. Environmental Conditions

The microgreen cultivation system was installed in a reach-in environmental growth chamber (Percival E41L2, Perry, Iowa) located in the Space Station Processing Facility at NASA Kennedy Space Center, Florida (located at sea level with atmospheric pressure of ~101 kPa). The chamber air temperature, relative humidity (RH), and CO₂ setpoints were maintained at 23 °C, 50%, and 3000 ppm, respectively, for all tests. These set points were chosen to simulate an environment similar to that of the ISS cabin air.

Un-sanitized seeds were sown on a sterilized hemp mat. The system was automatically irrigated several times daily with a modified 0.5 X Hoagland's nutrient solution, pH 5.8-6.5 and 1200 µS cm⁻¹ electrical conductivity (EC). Trays were covered with a transparent lid for 2-3 days to promote germination.

Lighting was provided by OSRAM LED grow lights (Phytofy RL, Munich, Germany). The photosynthetic photon flux density (PPFD) target was 150 µmol m⁻² s⁻¹, averaged over the entire canopy, with a 16-h (16 h light/8 h dark) photoperiod. Spectral quality consisted of 23% blue (400-500 nm), 27% green (500-600 nm), and 50% red (600-700 nm), using a combination of 450 nm, white (2700 K), 630 nm, and 660 nm LEDs. To minimize lighting hotspots and improve light distribution, a light diffusion acrylic panel (Curbell Plastics, Largo, FL) was installed over the surface of the LED fixture.

D. Crop Assessments

General appearance was monitored qualitatively throughout the 10 day experiment.

At 10-14 days after planting (DAP), the microgreens were harvested. Average canopy height (collected at three random locations), total harvest fresh mass, and presence of true leaves were recorded. Three subsamples were collected and oven dried at 70 °C for 72 hours, then weighed to collect dry mass.

E. Nutritional Analysis

Microgreens were harvested and frozen at -80 °C until they were shipped to a commercial laboratory (Eurofins Nutritional Analysis Center, Des Moines, IA), where they were analyzed for proximate composition (ash, calories, carbohydrates, fat, moisture, protein), mineral content (calcium, iron, magnesium, phosphorous, potassium), and vitamin content (B1, C, K).

F. Sensory Analysis

Microgreens were shipped live (unharvested and still attached to hemp mat) in coolers containing cold packs overnight from Kennedy Space Center (KSC) to Johnson Space Center (JSC), where formal sensory evaluation was conducted using the untrained volunteers at the Space Food Systems Laboratory sensory evaluation center. Each sample was rated on appearance, color, aroma, flavor, texture, and overall acceptability on a 9-point hedonic scale, where 1 corresponds to "dislike extremely" and 9 corresponds to "like extremely". Just About Right (JAR) measured the appropriateness of tenderness, bitterness, crispiness, nuttiness, sweetness, and aromatics on a 5-point scale. Example: 1=not tender at all, 2=not tender enough, 3=just about right, 4=somewhat too tender, 5=much too tender.

Due to ongoing issues with the COVID-19 pandemic, there were occasions where only an informal testing could be completed. These data were collected in a laboratory setting with n < 10.

III. Results

G. Harvest Metrics and Crop Morphology

Under these environmental conditions and when grown in our system, harvesting at 10 DAP was adequate for the majority of the crops. At 10 DAP, the majority of the seeds had germinated, a sufficient lawn had formed and true leaves were not present. One caveat is that the pea cultivars could have been harvested at 7 or 8 days, which would have produced a more tender, less fibrous product.

While we were ultimately successful, cantaloupe microgreens were extremely difficult to grow in this system. The vining nature of this crop prevented adequate root penetration into the hemp mat, often requiring the seeds to be weighed down during the germination phase. Even when grown in other media types (i.e. arcillite), roots grew up and out, along the surface of the media. It appears that this seed type is more easily cultivated in soil-based media, planted deeply (**Figure 2**).



Figure 2. Cantaloupe microgreens grown in arcillite (left) and hemp mat (right).

Horticulturally, the easiest crops to grow were *Brassicacae* (mustard family). Aspects that contributed to this ease included high and quick germination rates, seeds that did not harbor damaging endophytic microorganisms, an early root system which could easily penetrate the hemp matting, and a plant height that allowed easy separation from the hemp mat at harvest. Varieties with endophytic fungus issues included orach and sunflower cultivars. The fungus from orach was so invasive that the newly germinated seedlings were overcome, and mostly failed to thrive (**Figure 3**). Sunflower, on the other hand, often exhibited fungus on the seed coat that didn't seem to affect plant growth overmuch, but was considered unsightly and would be problematic for consumption (**Figure 4a**). Several varieties, such as alfalfa and wasabi arugula, were so short that they could not be easily harvested (**Figure 5**). The average heights of these plants were 19 and 20 mm, respectively. Additionally, the seeds of wasabi arugula were very small and hard to handle and sow.

Another issue often observed with large-seeded microgreens was the seedling's inability to shed its seed coat. Buckwheat shoot seeds maintained the hard, black seed coat, whereas buckwheat groats were hulled. Groats are the hulled kernels of cereal grains. Because of this, microgreens from groats were devoid of the seed coat (**Figure 4, b/c**), and would be more favorable for consumption.

The shungiku varieties proved to be the lowest yielding, due to low germination rates. High yields were observed from trays of buckwheat shoots/groats (356.3 g and 303.7 g), mung beans (339.4 g), and 'Mammoth Melting Sugar' peas (338.8 g). It should be noted that these are all large seed types, requiring between 56 to 133 g of seeds per tray to achieve these yields (**Table 5**).

Good germination and seedling establishment, absence of excessive fungus, and yield were all factors when choosing cultivars to down select. Images of down selected microgreens can be seen in **Figure 6** and **Figure 7**. Cultivar names, planting density, and resulting yields can be found in **Table 5**, where down selected cultivars used during additional testing are also highlighted.



Figure 3. Orach microgreens overcome with fungus.

H. Nutritional Analysis Results

Nutritional analysis data can be seen in **Table 1** and **Table 2**. Pea fruit (pods and seeds) are a good source for protein. They have up to 5% protein on fresh weight basis¹³, which we also observed in the microgreen version. 'Dwarf Grey Sugar' pea had the most protein of all types (5.88%), with Dun Pea (4.25%) and 'Mammoth Melting Sugar' pea (4.19%) coming in second and third, respectively. 'Dwarf Grey Sugar' pea was also the most calorically dense, with 57 kcal/100 g, and was one of two cultivars that contained the highest amount of phosphorus (0.089%).

Cilantro microgreens contained the highest amount of carbohydrates (7.65%), crude fat (1.09%), and potassium (0.707%), and were the other cultivar tied with ‘Dwarf Grey Sugar Pea’ for the highest amount of phosphorus (0.089%). The only standout amongst the *Brassicaceae* was white kohlrabi, which contained the highest amount of Ca (0.153%). While they did take a back seat to peas and other microgreen types, it must be noted that the microgreen versions of these *Brassicaceae* contain significantly more nutrients than their mature counterparts. For example, they can be more calorically dense. Collards, kohlrabi, and pac choi microgreens contain 9%, 41%, and 77% more calories than their full grown counterparts, respectively.¹⁴⁻¹⁶ Vitamin C content in Cressida cress exceeded all other varieties, with the cultivar containing 10.7 mg/100 g. High iron levels in astronauts after prolonged exposure to microgravity have been implicated in accelerating bone loss.¹⁷ Iron concentration was low in all microgreens tested (**Table 2**).

Table 1. Microgreen proximate and vitamin content. Values (fresh mass) of proximate and vitamin content of eighteen cultivars grown in controlled environment.

Cultivar	Proximate Tests						Vitamin		
	Ash	Calories	Carbohydrates	Crude Fat	Moisture	Protein	B1	C	K
	(%)	(kcal/100 g)	(Calculated-%)	(%)	(%)	(%)	(mg/100 g)	(mg/100 g)	(µg/g)
Mammoth Melting Sugar Pea	0.92	35	3.34	0.55	91.0	4.19	0.161	3.50	0.91
Cantaloupe	1.07	31	4.58	0.34	91.7	2.31	0.041	< 0.44	0.87
Shiso	0.73	31	4.18	0.43	92.1	2.56	0.068	< 0.44	0.57
Grey Striped Sunflower	0.89	43	7.15	0.67	89.1	2.19	0.073	< 0.44	0.72
Buckwheat Groats	0.68	46	7.10	0.62	88.6	3.00	0.138	3.75	0.20
Rutabaga	0.91	29	4.63	0.55	92.6	1.31	0.067	15.50	0.38
White Kohlrabi	1.45	34	5.49	0.60	90.9	1.56	0.050	11.80	0.18
Dun Pea	0.78	47	6.22	0.55	88.2	4.25	0.181	3.46	0.84
Chia	1.21	33	5.22	0.43	91.2	1.94	0.057	< 0.44	0.14
White Stem Pac Choi	1.13	23	3.60	0.39	93.5	1.38	0.040	2.26	0.10
Mung Beans	1.14	38	4.76	0.54	90.0	3.56	0.114	0.50	0.21
Cressida Cress	1.48	42	6.00	0.83	89.0	2.69	0.073	10.70	0.23
Dwarf Grey Sugar Pea	0.90	57	6.67	0.75	85.8	5.88	0.300	1.98	0.60
Persian Cress	1.44	36	5.07	0.56	90.3	2.63	0.071	3.75	0.51
Cilantro	1.97	53	7.65	1.09	86.1	3.19	<0.050	< 0.44	2.49
Daikon Radish	0.70	35	3.88	0.84	91.7	2.88	0.150	1.03	2.65
Collards	0.74	35	4.98	0.69	91.4	2.19	0.100	0.66	1.83
Wasabi Mustard	1.10	34	4.99	0.53	91.0	2.38	<0.050	0.83	2.39

Table 2. Microgreen mineral content. Values (fresh mass) of mineral content of eighteen cultivars grown in controlled environment.

Cultivar	Mineral Content					
	Ca (%)	Fe (%)	Mg (%)	P (%)	K (%)	S (%)
Mammoth Melting Sugar Pea	0.069	0.0007	0.026	0.065	0.337	0.05
Cantaloupe	0.129	0.0009	0.067	0.086	0.296	0.04
Shisio	0.085	0.0038	0.035	0.061	0.194	0.03
Grey Striped Sunflower	0.095	0.0045	0.053	0.060	0.289	0.05
Buckwheat Groats	0.053	0.0019	0.061	0.088	0.179	0.05
Rutabaga	0.111	0.0004	0.041	0.062	0.256	0.08
White Kohlrabi	0.153	0.0004	0.047	0.077	0.448	0.14
Dun Pea	0.073	0.0012	0.026	0.064	0.278	0.05
Chia	0.148	0.0109	0.055	0.050	0.338	0.03
White Stem Pac Choi	0.103	0.0004	0.031	0.051	0.385	0.09
Mung Beans	0.123	0.0007	0.048	0.057	0.394	0.04
Cressida Cress	0.085	0.0008	0.038	0.081	0.584	0.12
Dwarf Grey Sugar Pea	0.064	0.0012	0.031	0.089	0.289	0.05
Persian Cress	0.078	0.0006	0.037	0.112	0.524	0.11
Cilantro	0.117	0.0023	0.061	0.089	0.707	0.03
Daikon Radish	0.092	0.0006	0.046	0.066	0.153	0.13
Collards	0.101	0.0006	0.040	0.072	0.161	0.10
Wasabi Mustard	0.107	0.0007	0.045	0.071	0.338	0.10

I. Organoleptic Results

Sensory panelists were presented with washed samples and asked to rate their taste using a 9-point hedonic scale, and a “just about right” (JAR) scale that measures the appropriateness of the level of a specific attribute. The results of the panel assessments and standard deviations are shown in **Table 3** and **Table 4**.

Fifteen of the eighteen microgreens received an overall rating of 6.0 or higher, which is considered good. Cantaloupe received the highest overall score (7.67), with several tasters commenting on its sweetness and that it smelled *and* tasted just like mature cantaloupe fruit. *Brassic*as ‘White Stem’ pac choi (7.40), wasabi mustard (7.36), and rutabaga (7.31) achieved the next highest overall ratings after cantaloupe. ‘White Stem’ pac choi was enjoyed due to its milder flavor that wasn’t as overpowering as other microgreens. On the other hand, ‘Wasabi’ mustard was also enjoyed due to the spicy kick that it provided. Tasters noted that it would pair well with other leafy mixes, or as a topping on other food.

The lowest rated microgreens were shiso (5.67), Cressida cress (5.27), and chia (4.59). Although shiso had great texture and appearance, several tasters reported that it tasted and smelled like black licorice and was soapy with a medicinal finish. The data reflect these comments, with shiso scoring 7.2 in texture, 7.83 in appearance, and 5.67 in flavor. Cressida cress did not fare well during its journey to JSC. Tasters did not enjoy the yellow leaves; therefore, it received the lowest score in appearance of all the microgreens (6.67). While several panelists claimed that the spiciness of Cressida cress was similar to wasabi mustard, it was not as well received. It appears there was also a bitterness that accompanied the spicy, which made it less appealing. The data reflect these comments, with Cressida cress receiving a 4.0 for bitterness (somewhat too bitter), while wasabi mustard received a 3.16 (just about right) (**Table 4**). Chia’s texture was lackluster and scored only 5.89. Tasters reported that a few of the leaves had turned

black and were very unappealing. This is most likely due to the short stature of the microgreens, which keeps the leaves too close to the wet hemp mat during transportation. Its bitterness also proved over-powering.

Table 3. Summary of microgreen sensory analysis attributes judged on a 9-point hedonic scale. Values are means, with a value of 6 generally considered acceptable. ** denotes informal data during COVID when full panels could not be conducted.

Cultivar	Attribute					
	Overall	Appearance	Color Intensity	Aroma	Flavor	Texture
Buckwheat Groats	6.65	7.26	7.35	6.57	6.57	6.61
Cantaloupe	7.67	7.97	8.17	6.90	7.70	7.83
Chia	4.59	6.78	7.11	5.59	4.11	5.89
Cilantro	6.76	7.24	7.48	7.16	6.56	7.16
Collards	6.74	7.78	7.78	6.26	6.57	7.39
Cressida Cress	5.27	6.67	6.37	5.50	5.07	7.07
Daikon Radish	6.96	8.16	8.36	5.88	6.68	8.12
Dun Pea	7.13	8.00	8.13	6.35	7.09	7.74
Dwarf Grey Sugar Pea	7.19	7.96	8.11	6.67	7.15	7.59
Grey Striped Sunflower	6.83	7.87	8.00	6.52	6.52	7.39
Mammoth Melting Sugar Pea	6.94	7.77	8.04	6.58	6.98	7.45
Mung Beans **	7.13	7.75	7.88	6.88	7.00	7.75
Persian Cress **	6.82	7.82	7.82	7.09	6.64	7.73
Rutabaga	7.31	8.00	8.04	6.50	7.08	7.81
Shiso	5.67	7.83	7.97	6.67	5.67	7.20
Wasabi Mustard	7.36	7.88	8.00	6.44	7.00	7.64
White Kohlrabi	6.85	7.44	7.70	5.89	7.04	7.44
White Stem Pac Choi	7.40	8.24	8.28	6.44	7.32	8.00

Table 4. Summary of microgreen Just About Right attributes judged on a 5-point scale. Values are means. ** denotes informal data.

Cultivar	JAR Attributes					
	Tenderness	Bitterness	Crispiness	Nuttiness	Sweetness	Aromatics
Buckwheat Groats	3.26	3.26	2.65	2.74	2.61	2.91
Cantaloupe	3.10	3.10	2.93	2.90	2.73	2.73
Chia	3.07	4.15	2.74	2.93	2.11	2.78
Cilantro	3.16	3.28	2.56	2.72	2.56	3.08
Collards	3.00	3.26	2.78	2.87	2.52	2.61
Cressida Cress	3.00	4.03	2.87	3.03	2.13	3.00
Daikon Radish	3.04	3.56	2.88	2.64	2.40	2.52
Dun Pea	2.96	3.48	3.09	2.91	2.57	2.61
Dwarf Grey Sugar Pea	3.04	3.22	2.89	2.78	2.56	2.52
Grey Striped Sunflower	3.00	3.65	2.83	2.74	2.65	2.61
Mammoth Melting Sugar Pea	2.94	3.03	2.67	2.63	2.66	2.35
Mung Beans **	3.00	3.63	3.00	2.38	2.63	2.25
Persian Cress **	3.00	3.36	3.00	2.73	2.18	2.91
Rutabaga	3.00	2.88	2.88	2.58	2.58	2.38
Shiso	3.00	3.67	2.93	2.73	2.30	3.07
Wasabi Mustard	3.08	3.16	2.88	2.76	2.56	2.52
White Kohlrabi	3.00	3.07	2.78	2.81	2.70	2.22
White Stem Pac Choi	3.08	2.92	2.88	2.60	2.72	2.56

Discussion

Of the 18 cultivars down selected for nutritional and organoleptic analysis, only two (shiso and chia) would be withheld from future testing due to poor palatability. All other microgreens were well received by tasters. Issues with product quality often reduced palatability, rightfully so. The shelf life of microgreens is a major concern, even for commercial producers who have explored modified packaging strategies, temperature control, and calcium treatments to extend shelf life¹⁸. It should be noted that, while none of these strategies were utilized here, every effort was made to supply a high-quality product (shipping live and cold). Food safety testing, which was conducted on product that was freshly harvested, showed that the samples had higher microbial counts in comparison to other leafy greens grown in controlled environments at KSC. Decreasing product quality, such as wilting, dehydrating, and decay, raises an even higher concern for foodborne illness. Various strategies have since been explored to reduce the microbial and fungal load on microgreen products. Testing of cultivation under a blue-rich light spectrum, which decreases product height prior to harvest, and plant growth hardware that separates the root system from the foliage have proven to have some effect on the microbial load¹⁹. If used in conjunction with Prosan, a vegetable sanitizer that has been used by ISS crew members to sanitize produce grown in the Veggie facility, these strategies could provide a product with lower microbial and fungal loads. Microgreens are nutritional powerhouses. If chosen and cultivated correctly, they have the ability to supply substantial amounts of protein, vitamin C, B1, and K.

This raises the question of how many microgreens to produce at any given time in a space mission scenario. We suggest to grow and harvest single serve portions for consumption. Testing reported here advanced these microgreen crops to CRL 3. Additional testing reported by Hummerick et. al (2022), moves these crops to CRL 4. To further advance along the CRL, future testing should be in flight-forward hardware and include further food safety testing in this hardware.

Appendix

Table 5. Screened microgreens and the recommended planting density, total seed weigh planted, harvest point, and total fresh mass harvested. Down selected crops are highlighted. ND for cilantro. ND=No Data.

Crop	Scientific Name	Recommended Planting Density (g/4 x 4 in.)	Total Seed Weigh Planted/Trough (g)	Harvest DAP	Total FM (g/g seed)	Average Canopy Height (mm)	% Dry Mass
Alfalfa	<i>Medicago sativus</i>	1.5	10.5	10	30.4	19.0	9.5%
Arugula	<i>Eruca sativa</i>	1.0	7.0	10	86.1	45.3	7.0%
Arugula, Wasabi	<i>Diplotaxis eruroides</i>	0.8	5.6	10	77.7	26.0	ND
Basil, 'Dark Opal'	<i>Ocimum basilicum</i>	1.4	9.8	14	87.1	26.0	7.6%
Beet, 'Bulls Blood'	<i>Beta vulgaris</i>	3.5	24.5	14	64.6	38.3	12.5%
Broccoli	<i>Brassica oleracea italica</i>	1.0	7.0	10	80.7	63.7	7.6%
Brussel Sprouts	<i>Brassica oleracea</i>	0.9	6.3	10	43.1	22.0	10.3%
Buckwheat, Groats	<i>Fagopyrum esculentum</i>	8.0	56	10	303.7	120.0	7.7%
Buckwheat, OG	<i>Fagopyrum esculentum</i>	8.0	56	10	237.5	110.3	ND
Buckwheat, Shoot	<i>Fagopyrum esculentum</i>	8.0	56	10	356.3	137.7	ND
Cabbage, 'Mammoth Red Rock'	<i>Brassica oleracea capitata</i>	1.0	7.0	10	84.7	51.0	7.3%
Cabbage, Red	<i>Brassica oleracea capitata</i>	1.0	7.0	10	61.0	31.3	ND
Cantaloupe	<i>Cucumis melo</i>	3.0	21	14	40.0	39.0	10.1%
Cauliflower, 'Y Improved'	<i>Brassica oleracea botrytis</i>	1.0	7.0	10	72.4	46.3	8.4%
Chia	<i>Salvia hispanica</i>	1.0	7.0	14	87.7	57.0	8.2%
Chinese Cabbage	<i>Brassica rapa chinensis</i>	0.9	6.3	10	103.4	ND	6.5%
Collards, 'Vates'	<i>Brassica oleracea</i>	0.9	6.3	10	56.2	32.0	9.1%
Cress, Cressida	<i>Lepidium sativum</i>	0.9	6.3	10	58.1	39.0	8.6%
Cress, Persian	<i>Lepidium sativum</i>	1.0	7.0	14	65.8	36.0	11.7%
Cress, 'Upland'	<i>Barbarea verna</i>	1.0	7.0	14	57.6	18.0	8.3%
Dandelion, Red	<i>Chichorium intybus</i>	1.0	7.0	10	71.0	41.3	9.0%
Fennel	<i>Foeniculum vulgare</i>	2.0	14.0	14	64.0	73.3	12.1%
Golden Frills Mustard	<i>Brassica junco</i>	0.8	5.6	10	69.9	46.3	7.8%
Kale, 'Dwarf Siberian'	<i>Brassica oleracea acephala</i>	1.0	7.0	10	102.3	58.7	7.1%
Kale, 'Red Russian'	<i>Brassica napus</i>	1.0	7.0	10	97.3	65.3	9.9%
Kale, Toscano	<i>Brassica oleracea</i>	1.0	7.0	10	55.4	ND	8.9%
Kale, 'Vates Blue Scotch Curled'	<i>Brassica oleracea acephala</i>	1.0	7.0	10	85.8	46.7	9.0%
Kohlrabi, Purple	<i>Brassica oleracea</i>	0.9	6.3	10	38.7	35.0	11.4%
Kohlrabi, White	<i>Brassica oleracea</i>	1.0	7.0	14	100.5	69.7	7.0%
Komatsuna	<i>Brassica rapa perviridis</i>	1.0	7.0	10	98.8	ND	7.6%
Lentils	<i>Lens culinaris</i>	7.0	49.0	14	90.9	176.0	16.4%
Lettuce Mix, Mucelum	<i>n/a</i>	0.9	6.3	10	48.8	35.0	8.5%
Mizuna	<i>Brassica rape</i>	0.9	6.3	10	67.9	56.3	7.9%
Mizuna, 'Red Kingdom'	<i>Brassica japonica</i>	1.0	7.0	10	61.0	51.0	7.8%
Mung Beans	<i>Vigna radiata</i>	11.0	77.0	14	339.4	191.0	10.1%
Mustard, 'Garnet Giant'	<i>Brassica juncea</i>	0.9	6.3	10	108.2	ND	5.9%
Mustard, 'Scarlet Frills'	<i>Brassica juncea</i>	0.8	5.6	10	88.0	ND	6.5%
Mustard, 'Wasabi'	<i>Brassica juncea</i>	1.0	7.0	10	65.5	47.3	7.2%
Orach	<i>Atriplex hortensis</i>	2.0	14.0	14	13.0	32.0	ND
Pac Choi, Red	<i>Brassica rape chinensis</i>	1.0	7.0	10	46.6	39.7	38.6%
Pac Choi, 'Rosi'	<i>Brassica rapa chinensis</i>	1.0	7.0	10	112.9	ND	6.2%
Pac Choi, White Stem	<i>Brassica rapa chinensis</i>	1.0	7.0	10	152.5	58.0	5.7%
Pac Choi, White Stem Extra Dwarf	<i>Brassica rapa</i>	1.0	7.0	14	177.5	70.0	7.0%
Pea, Dun	<i>Pisum sativum</i>	17.0	119.0	14	233.2	199.0	8.3%
Pea, 'Dwarf Grey Sugar'	<i>Pisum sativum</i>	17.0	119.0	14	100.5	126.0	12.7%
Pea, Mammoth Melting Sugar'	<i>Pisum sativum</i>	19.0	133.0	14	338.8	260.0	7.0%
Quinoa	<i>Chenopodium quinoa</i>	2.0	14.0	14	ND	ND	ND
Radish, Daikon	<i>Raphanus sativus</i>	2.0	14.0	10	202.0	82.3	ND
Radish, Daikon (white)	<i>Raphanus sativus</i>	2.0	14.0	10	150.3	99.7	17.4%
Radish, 'Red Rambo'	<i>Raphanus sativus</i>	2.0	14.0	10	134.9	88.3	6.9%
Rutabaga	<i>Brassica napobrassica</i>	2.3	16.1	10	167.8	65.0	6.2%
Shiso	<i>Perilla frutescens</i>	2.0	14.0	14	135.6	40.0	7.5%
Shungiku	<i>Glebionis coronaria</i>	2.0	14.0	10	20.9	19.7	10.8%
Shungiku, Broadleaf	<i>Glebionis coronaria</i>	2.0	14.0	10	17.1	15.7	13.7%
Sunflower, 'Black Oil'	<i>Helianthus annuus</i>	10.0	70.0	10	185.0	72.3	ND
Sunflower, 'Grey Striped'	<i>Helianthus annuus</i>	7.0	49.0	10	217.1	96.7	8.2%
Swiss Chard, Yellow	<i>Beta vulgaris</i>	3.5	24.5	14	45.5	53.3	11.6%
Tatsoi	<i>Brassica rapa</i>	0.9	6.3	10	46.8	46.0	10.4%
Turnip, 'Purple Top White Globe'	<i>Brassica rapa</i>	1.0	7.0	10	116.7	57.0	ND



Figure 4. 'Black Oil' sunflower (a) with attached seed coats exhibiting fungal growth, (b) buckwheat shoots with attached seed coats, red arrow, and (c) buckwheat groats, devoid of attached seed coats.



Figure 5. Wasabi arugula microgreens, with an average canopy height of 20 mm, grown on hemp mat.



Figure 7. Eight of the eighteen down-selected microgreen cultivars. Rutabaga, white Kohlrabi, ‘Grey Striped’ sunflower, Persian cress, shiso, Daikon radish, Cressida cress and collards.



Figure 6. Ten of the eighteen down-selected microgreens. ‘Mammoth Melting Sugar’ pea, Dun pea, ‘Dwarf Grey Sugar’ pea, chia, Wasabi mustard, buckwheat groats, cantaloupe, mung bean, ‘White Stem’ pac choi, and cilantro.

References

- ¹Cooper, M., Perchonok, M., Douglas, G.L., Initial Assessment of the nutritional quality of the space food system over three years of ambient storage. *NPJ Microgravity*, 3:17. 2017.
- ²Perchonok, M.H., Cooper, M.R., and P.M. Catauro. 2012. Mission to mars: food production and processing for the final frontier. *Ann. Rev. Food Sci. Technol.* 3: 311-330.
- ³MacElroy, R.D., M. Kliss, and C. Straight. 1992. Life support systems for Mars transit. *Adv. Space Res.* 12(5):159-166.
- ⁴Kliss, M., Heyenga, A.G., Hoehn, A., Stodieck, L.S. "Recent Advances in Technologies Required for a 'Salad Machine'". *Advances in Space Research*, Volume 26, Issue 2, p. 263-269.
- ⁵Massa, G.D., Wheeler, R.M., Morrow, R.C., Levine, H.G., Growth Chambers on the International Space Station for Large Plants", *Acta Horticulturae*, 1134:215-221; DOI 10.17660. 2016.
- ⁶Tredwell, D.D., Hochmuth, R., Landrum, L., and Laughlin, W. "Microgreens: A New Specialty Crop". UF IFAS Extension. Document HS1164. 2020.
- ⁷Weber CF. Broccoli Microgreens: A Mineral-Rich Crop That Can Diversify Food Systems. *Front Nutr.* 2017 Mar 23;4:7. doi: 10.3389/fnut.2017.00007. PMID: 28386543; PMCID: PMC5362588.
- ⁸Yanqi Zhang, Zhenlei Xiao, Emily Ager, Lingyan Kong, Libo Tan. "Nutritional quality and health benefits of microgreens, a crop of modern agriculture" *Journal of Future Foods*. Volume 1, Issue 1. 2021. Pages 58-66.
- ⁹Xiao, Z., Codling, E.E., Luo, Y., Nou, X., Lester, G.E., and Wang, Q. 2016. Microgreens of Brassicaceae: Mineral composition and content of 30 varieties, *Journal of Food Composition and Analysis*, Volume 49, P87-93.
- ¹⁰Sun J, Xiao Z, Lin LZ, Lester GE, Wang Q, Harnly JM, Chen P. Profiling polyphenols in five Brassica species microgreens by UHPLC-PDA-ESI/HRMS(n). *J Agric Food Chem.* 2013 Nov 20;61(46):10960-70. doi: 10.1021/jf401802n. Epub 2013 Nov 5. PMID: 24144328; PMCID: PMC3915300.
- ¹¹Riggio, G. M., Jones, S. L., & Gibson, K. E. 2019. Risk of human pathogen internalization in leafy vegetables during lab-scale hydroponic cultivation. *Horticulturae*, 5, 1–22.
- ¹²Romeyn, M.W., Spencer, L.E., Massa, G.D., and Wheeler, R.M. Crop Readiness Level (CRL), a scale to track progression of crop testing for space, 2019. 49th International Conference on Environmental Systems Conference, ICES-2019-342.
- ¹³U.S. Department of Agriculture, Peas, green, raw. FoodData Central. <https://fdc.nal.usda.gov/fdc-app.html#/food-details/170419/nutrients>. FDC Published 4/1/2019.
- ¹⁴U.S. Department of Agriculture, Collards, raw. FoodData Central. <https://fdc.nal.usda.gov/fdc-app.html#/food-details/170406/nutrients>. FDC Published 4/1/2019.
- ¹⁵U.S. Department of Agriculture, Kohlrabi, raw. FoodData Central. <https://fdc.nal.usda.gov/fdc-app.html#/food-details/168424/nutrients>. FDC Published 4/1/2019.
- ¹⁶U.S. Department of Agriculture, Cabbage, Chinese (pak-choi), raw. FoodData Central. <https://fdc.nal.usda.gov/fdc-app.html#/food-details/170390/nutrients>. 4/1/2019.
- ¹⁷Swart, S.R. J.L. Morgan and S.M. Smith. 2013. Iron status and its relations with oxidative damage and bone loss during long-duration space flight on the International Space Station. *Am. J. Clin. Nutr.* 98: 217-223.
- ¹⁸Turner, E.R., Luo, Y., and Nuchanan, R.L. 2020. Microgreen nutrition, food safety, and shelf life: a review. *Journal of Food Science*, Vol. 85. Issue 4. P870-882.
- ¹⁹Hummerick, M. E., Curry, A.B., Gooden, J.L., Sporn, C.J., Fischer, J.A., and Spencer, L.E. 2002. The Microbiology of Microgreens Grown in Controlled Environment Chambers Under ISS Conditions. 51st International Conference on Environmental Systems, ICES-2022-267.