Dwarf Tomato and Pepper Cultivars for Space Crops

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Several dwarf tomato and pepper varieties were evaluated under ISS-simulated growth conditions (22°C, 50% RH, 1500 ppm CO₂, and 300 µmol m⁻² s⁻¹ of light for 16 h per day) with the goal of selecting those with the best growth, nutrition, and organoleptic potential for use in a pick and eat salad crop system on ISS and future exploration flights. Testing included six cultivars of tomato (Red Robin, Scarlet Sweet 'N' Neat, Tiny Tim, Mohamed, Patio Princess, and Tumbler) and six cultivars of pepper (Red Skin, Fruit Basket, Cajun Belle, Chablis, Sweet Pickle, and Pompeii). Plants were grown to an age sufficient to produce fruit (70 to 106 days for tomato and 109 days for pepper). Tomato fruits were harvested when they showed full red color, beginning ca. 70-days age and then at weekly intervals thereafter, while peppers were grown until numerous fruits showed color and all fruits (green and colored) were harvested once at the end of the test. Plant sizes, yields, and nutritional attributes were measured and used to down-select to three cultivars for each species. In particular, we were interested in cultivars that were short (dwarf) but still produced high yields. Nutritional data included elemental (Ca, Mg, Fe, and K) composition, vitamin K, phenolics, lycopene, anthocyanin, lutein, and zeaxanthin. The three down-selected cultivars for each species were evaluated for sensory attributes, including overall acceptability, appearance, color intensity aroma, flavor and texture. The combined data were compared and given weighting factors to rank the cultivars as potential candidates for testing in space. For tomato, the ranking was 1) cv. Mohamed, 2) cv. Red Robin, and 3) cv. Sweet N' Neat. For pepper, the ranking was 1) cv. Pompeii, 2) cv. Red Skin, and 3) cv. Fruit Basket. These rankings are somewhat subjective but provide a good starting point for conducting higher fidelity testing with these crops (e.g., testing with LED lighting similar to the Veggie plant unit), and ultimately conducting flight experiments.

Nomenclature

BDL	=	Below Detectable Limit
DAP	=	Days After Planting
NASA	=	National Aeronautics and Space Administration
ORAC	=	Oxygen Radical Absorbance Capacity
PAR	=	Photosynthetically Active Radiation
TE	=	Trolox Equivalents (related to ORAC)

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I. Introduction

The capability to grow nutritious, palatable food for crew consumption during spaceflight becomes increasingly important as we move toward long-duration, exploration-class missions. Critical nutrients may degrade in the current prepackaged space-food system and potentially fail to meet the shelf-life requirements of long-duration mission scenarios¹. Dietary delivery of these nutrients in the form of fresh vegetables is preferred due to synergistic benefits of naturally-occuring phytochemicals in whole-food delivery, slower degradation of nutrients when consumed as a food component, and the increased likelihood of crew compliance with eating versus taking capsules ^{2,3,4,5,6}. Implementation of a "pick-and-eat" bioregenerative produce system in spaceflight has potential to supplement nutrition over long durations, while at the same time supporting crew psychosocial health through the introduction of fresh foods that provide increased variety, texture, flavors, and colors⁷. Even having plants growing and marking the passage of time, and adding plant tending activities to crew routines may have significant knowledge gap remains to determine the best cultivars to obtain acceptable, nutritious pick-and-eat foods for consumption in the spaceflight environment. At the same time, reduced-area container gardening has increased in popularity, and numerous new commercial vegetable varieties that can be productive in space-constrained environments are now available.

Candidate tomato and pepper cultivars were grown in controlled environment chambers and evaluated for cultivar specific horticultural performance under mission relevant conditions. Horticultural metrics such as seed germination rate, time to fruit maturity, plant stature, and yield were evaluated. In addition, the quality and characteristics of the fruit at harvest were assessed, including nutritional and phytochemical attributes. Based on these evaluations, three cultivars of pepper and three cultivars of tomato were down-selected. These cultivars were re-grown to maturity and fruit harvested and shipped to NASA's Johnson Space Center for organoleptic acceptability (taste) testing.

Specific Aims:

- 1. Identify dwarf tomato and pepper cultivars
- 2. Assess growth and horticultural parameters of these cultivars using mission relevant conditions
- 3. Down-select the best cultivars for compact size, high yield, acceptability, and good nutrition.

II. Materials and Methods

<u>Tomato</u>. An initial survey of commercially available tomato (*Solanum lycopersicum* L.) cultivars was made and 19 dwarf varieties were identified as potential candidates. Varietal characteristics (from seed providers) were reviewed and cvs. Red Robin, Scarlet Sweet 'N' Neat, Tiny Tim, Mohamed, Patio Princess Hybrid, and Tumbler were selected (Table 1). Four seeds were planted per pot with plants thinned to one per pot ca. 7-10 days after germination. Four plants of each cultivar were grown in 10-cm square pots with custom potting media in controlled environment chambers. The media consisted of 7:3 Fafard #2B (Conrad Fafard, Agawam, MA): un-sieved turface (arcillite, Profile Products, LLC, Buffalo Grove, IL) with Nutricote timed release fertilizer (18-6-8 type 180) (Florikan, Sarasota, IL) mixed at a ratio of 10 g fertilizer per L of dry media. Media was premixed with water until damp and pots were filled. Beginning at 60 days after planting, all pots were watered three times per week with modified Hoagland nutrient solution to provide supplemental nutrients⁹. An automatic drip irrigation system was used, and timing of irrigations adjusted to ensure that water was not limiting.

Light was provided with high output, triphosphor fluorescent lamps with a 16 h light / 8 h dark photoperiod. Light at the plant canopy level was measured once per week, with a final average light reading of 309 μ mol m⁻² s⁻¹ (17.8 mol m⁻² d⁻¹ PAR). Environmental data were collected at 5-min intervals using OPTO 22 with PAC Project Pro Software (Temecula, CA) and in-house developed monitoring and control graphic user interface (L Koss, KSC Engineering Services Contract). Final average temperature, relative humidity and CO₂ concentrations for the initial tomato cultivar test were 22.0° C, 61.5% and 1449 ppm respectively.

The appearance of first flower bud, first flower, first fruit, and initial ripening were of tomatoes grown under the above conditions were recorded and are presented in Table 1. Ripe tomato fruit were harvested at 74, 81 and 89 days after planting. All remaining fruit were harvested at 89 days after planting.

Table 1. Days after planting (DAP) that first flower bud, flower, and fruit appeared and to initial ripening of six dwarf tomato cultivars. Final harvest was at 89 DAP.

Tomato Cultivar	Bud	Flower	Fruit	Ripening
Scarlet Sweet 'N' Neat	No data	31-36	36-42	66
Red Robin	23	31-36	41	66
Patio Princess	No data	36	41	64
Tumbler	24	28	41	64-66
Tiny Tim	No data	28	41	64-66
Mohamed	23	29-36	41	64-66

At harvest, plant height and two canopy diameter measurements (viewed from above) and fresh mass were recorded. Leaves and stems were oven dried at 70° C to calculate dry mass. Two intermediate harvests of ripe fruit were performed on 15 January (71 DAP), 22 January 2015 (78 DAP) with final harvest occurring on 29 January 2015 (85 DAP). Subsamples of fruit were then oven dried at 70°C, and dry mass taken in order to determine % moisture. Total number of ripe and unripe fruit were determined at the final harvest.

Cultivars were prioritized based short growth and high fruit yield, and fruit of the three top candidates were further analyzed for chemical and organoleptic attributes. Fruit were freeze dried for 2-3 weeks until completely dry. Freeze dried samples were ground with a Wiley Mill to pass through a 20-mesh screen and samples were refrozen at -80° C in 50 ml tubes until analysis. Dried tissue samples were analyzed for elemental content using inductively coupled plasma optical emission spectrometry (ICP-OES) and phytonutrient content using high pressure liquid chromatography (HPLC). Total phenolic and anthocyanin values were derived utilizing spectrophotometric methods. Vitamin K analyses were conducted by a commercial lab (Cornerstone Laboratories, LLC Memphis, TN).

A second planting of the three cultivars (Red Robin, Mohamed, and Sweet 'N' Neat) was initiated 4 May 2015 for organoleptic analysis. Plants were grown under the same environmental conditions used in the initial screening but with 10 plants/cultivar instead of four. Final average weekly light measurements were 324 μ mol m⁻² s⁻¹ (18.9 mol m⁻² d⁻¹ PAR). Final average temperature, relative humidity and CO₂ concentration for the second grow-out of tomato were 22.0° C, 60.3% and 1507 ppm respectively. Plants were grown and ripe fruit were harvested on 27 July 2015 (84 DAP) and shipped overnight for organoleptic / sensory analysis. Additional harvests of ripe fruit were performed on 6 August (94 DAP) and 13 August (101 DAP) with final harvest occurring on 18 August 2015 (106 DAP). Fresh weight (g), size, total number, and total harvest weight were recorded for all harvests. Subsamples of fruit were oven dried at 70° C, and dry mass taken in order to determine the percent moisture. Total number of ripe and unripe fruit were determined after the final harvest, as well as plant size.

Harvested tomato fruits for sensory analysis were placed in plastic clam-shell type food containers prior to packing into Styrofoam[™] boxes lined with 4°C cooling packs, and shipped to NASA's Johnson Space Center (JSC) for sensory analysis ¹⁰. Upon receipt, the produce was washed, photographed, and presented to a sensory panel. A total of 22 taste panelists were used by NASA's Advanced Food Technology team at JSC for organoleptic evaluation. Panelists were presented with samples of the washed produce, and asked to evaluate using at 9-point Hedonic Scale (1=dislike extremely, 2= dislike very much, 3=dislike moderately, 4=dislike slightly, 5=neither like nor dislike, 6=like slightly, 7=like moderately, 8=like very much, 9=like extremely) for overall acceptability, appearance, color intensity, aroma, flavor and texture.

<u>Pepper</u>. Dwarf peppers were identified using the same criteria as dwarf tomatoes, with six commercial cultivars selected for initial testing (Red Skin, Fruit Basket, Cajun Belle, Chablis, Sweet Pickle, and Pompeii) (Table 2).

Four plants of each cultivar were grown in 10-cm square pots starting on 5 November 2014 using the same custom potting media and fertilizer regimes detailed for tomato production. Plants were thinned to one per pot ca. 7-10 days after germination.

Light was provided with high output triphosphor fluorescent lamps with a 16 / 8 photoperiods, and intensity at the top of the plant canopy was measured weekly, with a final average of 316 μ mol m⁻² s⁻¹ (18.8 mol m⁻² d⁻¹ PAR). Environmental data were collected at 5-min intervals. Final average temperature, relative humidity and CO₂ values were 22.2° C, 55.8% and 1506 ppm respectively.

Plants were grown under the above conditions and appearance of first flower bud, first flower, first fruit, and initial ripening were recorded. Fruit were harvested at 109 (DAP, when many showed full color with some still being green (Table 2).

Table 2. Days after planting (DAP) that first flower bud, flower and first fruit for six dwarf pepper cultivars.
All plants were harvested at 109 DAP.

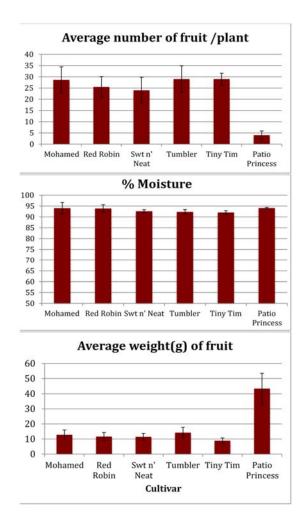
Tomato Cultivar	Bud	Flower	Fruit
Red Skin	36	47-51	52-55
Fruit Basket	36	45-50	52-55
Cajun Belle	36-38	47-48	55
Chablis	40-43	54-57	57-64
Sweet Pickle	36-52	48-66	55-65
Pompeii	36-40	48-54	52-55

At final harvest (109 DAOP), plant height and two canopy diameter measurements (viewed from above) and plant fresh mass were recorded. Leaves and stems were oven dried at 70°C to calculate dry mass. Subsamples of fruit were then oven dried at 70°C, and dry mass taken in order to determine % moisture. Total number of colored and green fruit were determined at the final harvest.

Cultivars were prioritized based on compact growth and fruit yield. Fruit of the three top candidates were analyzed for chemical and organoleptic attributes. Fruit were freeze dried for 2-3 weeks until completely dry. Freeze dried samples were processed and analyzed as described for the tomato trials. Dried tissue samples were analyzed for elemental content using inductively coupled plasma optical emission spectrometry (ICP-OES) and phytonutrient content using high pressure liquid chromatography (HPLC). Total phenolic and anthocyanin values were measured utilizing spectrophotometry methods. Vitamin K analyses were conducted by a commercial lab (Cornerstone Laboratories, LLC Memphis, TN).

The three top cultivars (cvs. Fruit Basket, Pompeii and Redskin) were selected and grown for a second test planted beginning on 4 May 2015 for sensory analysis, but with 10 plants per cultivar instead of 4. Harvest procedures were identical to those for the first experiment with fully colored and any remaining green fruit harvested at 106 DAP. The environmental conditions of the second grow out for sensory analysis were similar to the first experiment, with weekly light measurements averaging 314 μ mol m⁻² s⁻¹ PAR (18.8 mol m⁻² d⁻¹ PAR). As with the first experiment, the environmental data were collected at 5-min intervals. Average temperature, relative humidity and CO₂ values were 21.7° C, 50.9% and 1522 ppm respectively. Relative humidity was lower for the second experiment due to a failure of the chamber humidification control system during the second half of the experiment. Fresh fruits were packaged and shipped over night to NASA JSC for sensory evaluation, similar to tomato.

III. Results and Discussion



A. Tomato

Figure 1. Average number of tomato fruit per plant, percent moisture, and individual fruit fresh mass of tomato cultivars. Data combined from three sequential harvests at 71, 78 and 85 DAP.

The number of fruit per plant, fresh weight, and percent moisture of each fruit were obtained for the first two harvests at 71 and 78 days after planting (Fig. 1). There were no significant differences between cultivars with regards to number of fruit per plant, with the exception of Patio Princess, which had < 5 fruit per plant. However, there were differences in fruit size, with Patio Princess having fruit averaging 40 g fresh mass / fruit, and Tiny Tim averaging 8 g / fruit, with the remaining varieties having fruit size ranging from 10-12 g / fruit. There were no significant differences in percent moisture between the cultivars, with all values between 92 and 94% moisture.

Representative fruit sizes and color are shown in Figure 2. All the varieties produced similarly sized fruit, with good shape and color. Patio Princess was a larger fruited cultivar, and as such, produced fewer fruit per plant.

The total fresh mass of ripe tomato fruit harvested per plant is shown in Fig. 3. This value is the sum of total fresh mass of ripe fruit harvested at 71, 78 and 85 days after planting. Unripe fruit were not included in these totals.

Based on the yield and fruit size data, the only cultivar to be eliminated from consideration was Patio Princess, due to the low number of larger fruit and occurrence of blossom end rot. Larger numbers of smaller sized fruit would be more conducive to "pick-and-eat" consumption in microgravity by multiple crew. At the final harvest, biometric data on plant height, width, and fresh mass were obtained. There were significant differences in the developmental morphology of the six dwarf tomato cultivars under mission relevant conditions (Figure 4). Patio Princess, Tiny Tim and Tumbler tended to be 'leggy' and were unable to support weight of fruit on the stems. In contrast, Red Robin, Scarlet Sweet 'N' Neat, and Mohamed were compact, and had consistently high fruit set.

Based on the morphological data, Patio Princess, Tiny

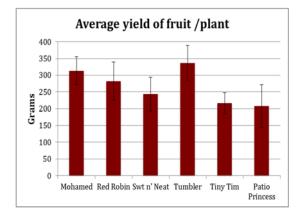


Figure 1. Average total fresh mass per plant of ripe tomato fruit. Bars indicate one standard deviation among plants.

was a trend towards slightly more fruit/plant in Mohamed than either Red Robin or Scarlet Sweet 'N' Neat.

Elemental analysis for Ca, Fe, K and Mg for ripe tomato fruit are shown in Table 3.

Iron (Fe): Iron is a necessary co-factor for many critical enzymatic systems, most notably as a cofactor for hemoglobin content in blood. However, high iron levels in astronauts after prolonged exposure to microgravity have been implicated in accelerating bone loss¹¹. Iron concentration was low in all three cultivars, with concentrations being below the level of detection for Red Robin and Mohamed.

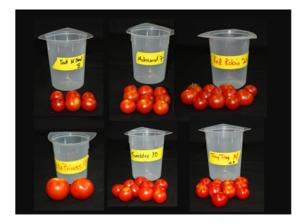


Figure 2. Fruit size and color of fruit harvested from six dwarf tomato varieties. The fruit are representative harvests from a single plant during the 2nd harvest (78 DAP) of the plants.

Tim and Tumbler were removed from further consideration, and Red Robin, Mohamed, and Sweet n' Neat were used for further testing. Yield data from the second grow out were similar to those of the initial cultivar down-select, with no statistically significant differences among cultivars with regard to number of fruit per plant, fruit size or percent moisture (Fig. 5). Although not statistically significant there



Figure 3. Morphology of six tomato cultivars grown in controlled environment chambers under ISS relevant conditions at 85 DAP. Average height ($cm \pm SD$) shown. All plants grown in 10-cm square pots.

	Ca	Fe	K	Mg		
Tomato Cultivar	$(\mu g/g)$					
Red Robin	947	BDL	24672 ^a	1440 ^a		
Mohamed	858	BDL	23740 ^{ab}	1375 ^{ab}		
Sweet n' Neat	781	8.09	22329 ^b	1210 ^b		

Table 3. Mean values (per gram dry weight) for elemental analysis of dwarf tomato cultivars. Different letters indicate significant differences within columns (p<0.05) (Tukey's multiple comparison test). BDL indicates below detectable level.

Potassium (K): Potassium is an essential electrolyte to maintain charge balance and energy transfer in cells. The crew diets are somewhat limited in Potassium and it was indicated as a high priority nutrient ¹². All species tested had high concentrations of K (>22 mg/ g dry mass). There was a statistical difference between cultivars with Red Robin being 9% higher than Scarlet Sweet 'N' Neat. These concentrations are comparable to those obtained with leafy greens ¹⁰.

Magnesium (Mg): Magnesium, along with Ca is necessary for the development and maintenance of bone structure ¹³. Mg concentrations ranged from 1.2 to 14 mg/g dry mass. As observed with K, there was a statistical difference between cultivars with the concentration of Mg in Red Robin being 15% greater than Scarlet Sweet 'N' Neat.

Phytonutrient and vitamin content were measured to establish a broader view of nutritional value. These included overall anti-oxidant potential, using ORAC as a general indicator of bioactive components, total phenolics, lycopene, anthocyanins, lutein, zeaxanthin, and Vitamin K (Table 4).

<u>Anti-Oxidant Potential</u>: All three dwarf tomato cultivars had relatively high ORAC levels. While it is generally believed that the presence of anthocyanins (see below), and the resultant increase in ORAC value, provides a measure of overall bioprotection in the diet, it has been difficult to establish the correlation *in vivo*¹⁴.

Lycopene: Lycopene is a lipophilic carotenoid found predominantly in tomatoes but also occurs in several other red fruits and vegetables. It is an anti-oxidant and a major carotenoid in the human blood. Lycopene protects against degenerative diseases by preventing oxidative damage to lipids, proteins and DNA¹⁵. The concentration of lycopene in dwarf tomato ranged from 34.99-42.02 mg/g dry mass. There were no statistical differences in lycopene levels between the three cultivars.

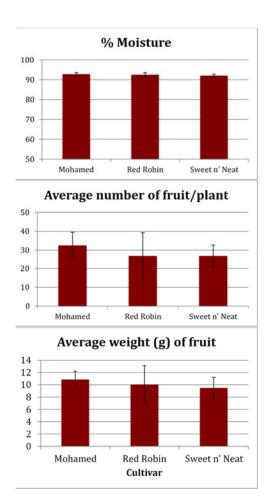


Figure 5. Percent moisture, average fruit number, and average fruit mass of three down-selected tomato cultivars. Data are means of four sequential harvests with error bars indicating one standard deviation.

	Phenolics	ORAC	Lycopene	Anthocyanin	Lutein	Zeaxanthin	Vit. K	
	μg/g	µmol TE/g	mg/g				mg/100g	
Tomato Cv.								
Red Robin	7.72ª	71.32ª	34.99	1.58	1.85	0.03	19.75ª	
Mohamed	7.17 ^a	74.70 ^a	36.98	2.00	1.77	0.03	18.85 ^{ab}	
Sweet'N'Neat	7.83ª	72.18 ^a	42.02	1.61	1.46	0.04	14.45 ^b	

Table 4. Mean values (in grams dry mass) of phytonutrient analysis of dwarf tomato. Different letters indicate significant differences within columns (p<0.05) (Tukey's multiple comparison test).

<u>Phenolics</u>: Phenolics are a large class of compounds with anti-oxidative activity and are primary chemical building blocks for many biologically active phytonutrients.

<u>Anthocyanin</u>: Anthocyanins are hydrophilic pigments (red, blue, and purple), which have very strong anti-oxidant potential. They have been suggested as biological countermeasures to radiation stress on long duration space missions¹⁶. Anthocyanins were detected in all cultivars, but there was no statistical difference among the three.

<u>Lutein and Zeaxanthin</u>: Lutein and zeaxanthin are carotenoids found in a number of fruit and vegetables, and are isomers, with lutein being the predominant form¹⁷. These two pigments accumulate in the retina of the eye and are believed to provide protection by filtering harmful UV wavelengths and dissipating excess visible light energy ¹⁷. Although there were numerical differences in the concentration of lutein between the cultivars, with Red Robin (1.85 mg/g) > Mohamed (1.77 mg/g) > Sweet 'N' Neat (1.61 mg/g), these differences were not statistically different. Zeaxanthin was only present at very low levels and again no differences were significant.

<u>Vitamin K</u>: Vitamin K has been correlated with reduction of arterial calcification and osteoporotic bone loss; it is a critical cofactor in the incorporation and retention of Ca⁺⁺ in bone structures¹⁸. Vitamin K is not available at adequate levels in the processed space food system^{1,12}. In the three cultivars tested, Red Robin had a statistically higher concentration of Vitamin K (19.75 mg/100g) than either Mohamed (18.85 mg/100g) or Sweet 'N' Neat (14.45 mg/100g).

While all three cultivars were very similar in overall nutrient content, Red Robin had statistically greater concentrations of K, Mg and Vitamin K than either Mohamed or Sweet 'N' Neat. All cultivars were much richer sources of phytochemicals then leafy greens on a mass basis.

<u>Sensory Analysis</u>. Only fruit from the initial harvest of tomato at 84 DAP (27 July 2015) were used for organoleptic analysis. Following collection of fresh mass data of the fruit, fruit from the three cultivars were packaged and sent via overnight delivery to the Sensory Evaluation Center at NASA's Johnson Space Center (Fig. 6).



Figure 4. Dwarf tomato fruit prior to harvest on 27 July 2015 (left) and selected fruit packed in containers for shipping for sensory analysis (right).

Advanced Food Technology (AFT) personnel coordinated the recruitment of 34 taste panelists and facilitated each evaluation session. Testing occurred in an isolated, controlled environment where panelists were instructed to evaluate each sample independently ¹⁹. The panelists were presented with samples of the washed produce, and asked to evaluate each item using at 9-point Hedonic Scale for overall acceptability, appearance, color intensity, bitterness, flavor, and intensity. The results of those panel assessments are shown in Table 5.

Table 5. Summary of sensory analysis results using 9-point hedonic scale for tomato fruit. Values are means \pm standard deviations (n = 34).						
Attribute	Red Robin	Mohamed	Sweet 'N' Neat			
Originall A compatibility	7 12 +1 75	$7(2 \pm 1.19)$	6.00 + 1.00			

Attribute	Red Robin	Mohamed	Sweet 'N' Neat
Overall Acceptability	7.12 ± 1.75	7.62 ± 1.18	6.88 ± 1.82
Appearance	8.15 ± 0.82	8.47 ± 0.56	8.03 ± 1.00
Color Intensity	8.00 ± 0.18	8.29 ± 0.72	8.06 ± 1.07
Aroma	6.44 ± 1.78	6.24 ± 1.58	6.18 ± 1.64
Flavor	7.06 ± 1.79	7.50 ± 1.44	6.65 ± 2.14
Texture	6.35 ± 2.44	7.91 ± 1.14	6.79 ± 2.16

These results indicate that the produce was generally acceptable amongst panelists, with all assessments being 6.0 (slightly like) or greater.

The panelists were also asked to evaluate the produce for sweetness, tartness and juiciness using a 5-point 'Just About Right' scale (1 = not nearly sweet/tart/juicy enough, 2 = not sweet/tart/juicy enough, 3 = just about right, 4 = too sweet/tart/juicy, 5 = much too sweet/tart/juicy). The results are presented in Table 6.

Table 6. Summary of sensory analysis results using a 5-point "Just About Right" scale for tomato fruit. Values
are means \pm standard deviations (n = 34).

Attribute	Red Robin	Mohamed	Sweet 'N' Neat
Sweetness	2.88 ± 0.77	2.82 ± 0.76	2.71 ± 0.87
Tartness	2.82 ± 0.72	2.62 ± 0.70	2.85 ± 0.78
Juiciness	3.44 ± 0.75	3.06 ± 0.24	3.06 ± 0.55

These analyses indicate that all three cultivars were well within acceptable ranges for all characteristics, and that variation among the cultivars was minimal.

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B. Pepper

The number of fruit, fresh weight of fruit, and total yield of pepper fruit per plant are shown in Figure 7. Cajun Belle and Sweet Pickle had the most fruit per plant (19 and 17 respectively), but these were also the smallest fruit, 10 and 9 g/fruit respectively. In contrast, the remaining four cultivars had significantly fewer fruit than these two cultivars with Redskin > Chablis > Fruit Basket > Pompeii. However, the fresh mass of the individual fruit was 3 to 5 times higher with Pompeii > Chablis> Redskin> Fruit Basket.

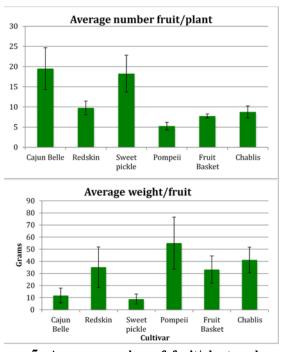


Figure 5. Average number of fruit/plant and average weight (mass) of individual fruit harvested from six dwarf pepper cultivars. Values for fresh mass of fruit are means (\pm SD) of at least five representative fruit from each of four plants (n \geq 20).

The consideration of both fruit number and total fruit mass per plant indicate Pompeii and Redskin had the highest yields (>250 g fruit/plant) and Sweet Pickle had the lowest (100 g fruit/plant). Overall ranking was Pompeii > Redskin > Chablis > Fruit Basket > Cajun Belle (Figure 8).

At harvest, the four plants of each cultivar were photographed for subsequent height and volume determination using image analysis. Plants at 109 DAP are shown in Figure 9.

Chablis was a compact plant, with a number of small fruit, which had taken on characteristic yellow or red pigmentation at harvest. The size of the plants was comparable to Pompeii, which had somewhat larger fruit, and transitioned from green to deep red when ripe. Fruit Basket and Redskin were similar in overall plant and fruit size, with Redskin having slightly higher yield per plant. Pepper fruits produced from Fruit Basket turn orange when ripe, while Redskin turns a deep red.

In contrast to the compact size of the first four cultivars, Cajun Belle was tall (>60 cm), with a large number of small fruit. These transitioned from green to deep red flesh when ripe. Sweet Pickle produced a large number of small, multi-colored fruit. While not as tall as Cajun Belle, it was 50% taller than the remaining four compact

varieties. Although Sweet Pickle was an attractive plant producing a large number of fruit, they were small, overall yield was low, and fruit did not test well in informal taste tests.

Cajun Belle and Sweet Pickle were not considered viable candidates for ISS Pick-and-Eat due to relatively large plant size and low yield, although elemental and nutrient analyses were still performed (Figures 8 and 9).

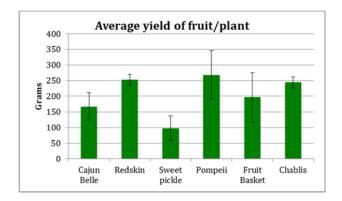


Figure 6. Average yields of fruit/plant from six dwarf pepper cultivars. Values indicate means of total fresh mass of fruit per plant \pm SD (n \geq 10).

Calcium (Ca): There was over a 2-fold difference in Ca concentration between the lowest (Red Skin) and highest (Chablis) cultivars. The two cultivars with the highest Ca levels, Cajun Belle and Chablis had levels significantly greater than the three cultivars selected for sensory analysis but did not meet the primary horticultural requirements for size and productivity. Pompeii had the highest Ca concentration of the varieties sent on for sensory analysis. It should be noted that pepper Ca concentrations were an order of magnitude or more lower than values found with leafy greens¹⁰.

Iron (Fe): Iron concentrations were below detection limits in all cultivars.

<u>Chemical analysis</u>. The results of the elemental analysis for Ca, Fe, K and Mg for the five pepper cultivars are shown in Table 7. The importance of these elements to the overall space food system was discussed previously. The cultivars in italics were ultimately selected for inclusion in sensory testing.



Figure 7. Comparison of height and morphology of six dwarf pepper cultivars grown in controlled environment chambers under mission relevant conditions. All plants grown in 10-cm square pots.

Table 7. Mean values (per gram dry mass) of elemental and nutrient analysis of dwarf pepper cultivars. Cv. Sweet Pickle was eliminated for further evaluation based on horticultural performance. Different letters indicate significant differences within columns (p<0.05) (Tukey's multiple comparison test). BDL indicates below detectable limit.

	Ca	Fe	K	Mg
Pepper Cultivar			$(\mu g/g)$	
Pompeii	1135 ^b	BDL	21154	1165 ^{ab}
Fruit Basket	872 ^b	BDL	21819	1085 ^b
Red Skin	793 ^b	BDL	19290	1203 ^{ab}
Cajun Belle	1297 ^{ab}	BDL	19904	1450 ^{ab}
Chablis	1898 ^a	BDL	20331	1500ª

Potassium (K): All pepper cultivars tested had high concentrations of K (>19 mg / g dry mass). There were no statistical differences in K concentration between the cultivars.

Magnesium (Mg): Pepper fruit Mg concentrations ranged from 1.0 to 1.5 mg / g dry mass. As observed with Ca there was a statistical difference between cultivars with the concentration of Mg in Chablis being nearly 50% higher than that of Fruit Basket. Chablis and Cajun Belle has the highest concentrations of Mg in fruit of the five cultivars tested, but these were not included in sensory tests because they failed to meet the threshold for horticultural acceptability. There was less than 10% difference in Mg concentration between Pompeii, Fruit Basket and Redskin, and this difference was not statistically significant.

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The results of phytonutrient and vitamin analyses for pepper fruit are shown in Table 8.

Table 8. Mean values (per gram dry mass) of phytonutrients of dwarf pepper fruit. Italics indicate cultivars sent for organoleptic testing. The cultivar Sweet Pickle was eliminated for further evaluation based on horticultural performance. Different letters indicate significant differences within columns (p<0.05) (Tukey's multiple comparison test).

	Phenolics	ORAC	Lutein	Zeaxanthin	Vit. K
Pepper Cultivar	µg∕g	µmol TE/g		mg/g	mg/100g
Pompeii	12.29	94.49 ^b	94.49 ^b	0.06 ^{ab}	113.00
Fruit Basket	12.56	128.06 ^{cb}	128.06 ^{cb}	0.03ª	116.5
Red Skin	13.27	111.24 ^{cb}	111.24 ^{cb}	0.08 ^b	112.5
Cajun Belle	14.42	150.97 ^a	150.97ª	0.07^{ab}	119.50
Chablis	14.39	137.22 ^{ac}	137.22 ^{ac}	0.04 ^{ab}	100.50

<u>Phenolics</u>. There were no differences in total phenolic content between the three pepper cultivars.

Antioxidant Potential. All three pepper cultivars selected for organoleptic testing had relatively high ORAC levels, with Cajun Belle having the highest, and Pompeii the lowest, values. Although Cajun Belle and Chablis had the highest ORAC values they were not selected for further sensory analysis because they failed to meet horticultural requirements. Fruit Basket and Red Skin had the highest values of the remaining three.

<u>Lutein and Zeaxanthin</u>. As with the other phytonutrients, there were statistical differences among cultivars for lutein and zeaxanthin, with Cajun Belle >Chablis>Fruit Basket>Red Skin>Pompeii for lutein, and Red Skin and Cajun Belle having the highest concentration of zeaxanthin. Despite these positive nutritional attributes, Cajun Belle was not selected for final consideration due to the large size of the plants.

<u>Vitamin K</u>. There were no statistical differences in Vitamin K among the pepper cultivars. Interestingly, Vitamin K content of unripe (green) fruit averaged 19 mg /100 g fresh mass greater than that of ripe fruit across all cultivars tested (data not presented).

Unlike tomato, pepper has a long period when it is edible that is not dependent upon the pigmentation of the tissue. For these studies, a fruit was considered ripe when it had started to show color other than green. At harvest the unripe (green) and ripe (showing color) fruit were analyzed separately for anthocyanin concentration (Table 9).

<u>Anthocyanin</u>. The concentration of anthocyanin in unripe green pepper fruit was low, and there were no statistical differences among the cultivars. Upon ripening, Red Skin and Cajun Belle had the highest concentration of anthocyanin, which was approximately 6 x that of the green fruit. Of the three cultivars selected for sensory analysis based on horticultural characteristics, there was no statistical difference in anthocyanin concentration between Pompeii and Redskin. They each had approximately 2 x the anthocyanin concentration of Fruit Basket, although in the case of Pompeii vs. Fruit Basket the 2x difference was not significant.

Table 9. Mean values (per gram dry mass) of anthocyanin concentration of ripe and unripe fruit of dwarf pepper. The cultivar Sweet Pickle was eliminated for further evaluation based on horticultural performance. Different letters indicate significant differences within columns (p<0.05) (Tukey's multiple comparison test).

	Anthocyanins		
	Unripe	Ripe	
Pepper	µg/g		
Cultivar			
Pompeii	0.29	3.91 ^{abcd}	
Fruit Basket	0.12	1.84ª	
Red Skin	0.71	4.12 ^d	
Cajun Belle	0.85	4.97 ^{bd}	
Chablis	0.53	3.34°	

consistent with results from the initial harvest, where Fruit Basket averaged 8 fruit per plant and Pompeii averaged 5. Pepper fruit were considered ripe if there was pigmentation (red, yellow, or orange) present on the skin.

There was an inverse relationship between fruit number and size of fruit, with Pompeii being significantly heavier (36 g/fruit) than Fruit Basket (22 g/fruit). Redskin was intermediate at just under 30 g/fruit. This is consistent with results from the initial test where fruit size for Pompeii > Redskin > Fruit Basket. Fruit from the 2nd harvest were smaller than those from 1st harvest however. This was likely due to multiple harvests used for the initial screening, which resulted in fewer but larger fruit at the final harvest. Also, because these plants were grown in 10-cm pots, their overall growth was likely restricted due to the pot size²⁰, which in turn might have affected fruit size and number.

Total yield of pepper fruit per plant is shown in Fig.11. As with the initial screening test, Pompeii had the highest total fresh mass per plant, followed by Redskin, then Fruit Basket. Pompeii yielded 265 g fresh mass per plant, followed by Redskin at 250 g, and Fruit Basket at 220 g per plant. Overall, cv. Pompeii produced the largest fruit mass and total yield per plant for the three down-selected cultivars used for sensory analysis.

<u>Sensory Analysis Results</u>. The averages of the sensory panel assessments for pepper fruit are shown in Table 10. These results indicate that the produce was generably acceptable amongst panelists,

The number of fruit per plant, the average fresh mass of individual fruit, and the percent moisture for each of the three candidate cultivars sent for organoleptic analysis is shown in Figure 10. There were no statistical differences in percent moisture among the three cultivars with Fruit Basket, Pompeii and Redskin each having 90% moisture content. There were significant differences in the number of ripe fruit per plant at 106 DAP between the three cultivars, with Fruit Basket having 8 and Pompeii 5. Redskin was intermediate with 7. These data are

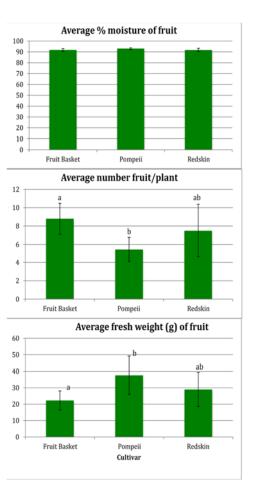


Figure 8. Average percent moisture, number of fruit per plant, and average weight (mass) per fruit for three dwarf pepper cultivars. Data represent means of 10 plants with error bars indicating one standard deviation.

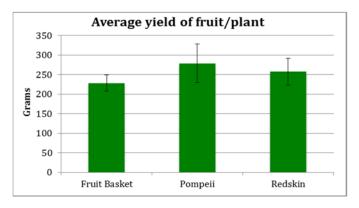


Figure 9. Fresh mass (g) of fruit per plant of three downselected dwarf pepper cultivars. Data represent means of n=10 plants with error bars indicating one standard deviation. as indicated by an average acceptability score of 6.0 or higher for each attribute. The only exception was with the cultivar Fruit Basket, which had a value less than 6.0 for flavor. Overall the panelist had a strong preference for Pompeii giving it the highest ratings on all factors followed by Red Skin. Scores indicate Fruit Basket was generally less appealing.

The panelists were also asked to evaluate the produce for astringency/bitterness, sweetness and juiciness using a 5 point Just About Right scale (1=not nearly bitter/sweet/juicy enough, 3=just right, 4=too bitter/sweet/juicy, 5=much too bitter/sweet/juicy). The results are presented in Table 11.

Table 10. Sensory analysis results pepper fruit using 9-point hedonic scale. Values are means (± one standard deviation, n=22).

Attribute	Pompeii	Red Skin	Fruit Basket
Overall Acceptability	7.91 ± 0.68	6.77±1.54	6.23 ± 1.82
Appearance	8.14 ± 1.08	7.09 ± 1.72	8.18 ± 0.96
Color Intensity	8.23 ±1.11	7.64 ± 1.92	8.27 ± 1.03
Aroma	7.59 ± 1.22	6.82 ± 1.44	6.82 ± 1.59
Flavor	7.73 ± 0.88	6.77 ±1.57	5.73 ± 2.00
Texture	8.27 ± 0.63	7.95 ± 1.00	7.82 ± 1.05

Table 11. Summary of Bitterness, Sweetness and Juiciness for pepper fruit using a 5-point "Just About Right" evaluation scale. Values are means (± one standard deviation, n=22).

Attribute	Pompeii	Red Skin	Fruit Basket
Astringency/ Bitterness	2.50 ± 0.96	3.00 ± 0.98	3.82 ± 0.73
Sweetness	2.68 ± 0.57	2.27 ± 0.77	1.95 ± 0.79
Juiciness	3.00 ± 0.31	3.00 ± 0.31	2.86 ± 0.35

These analyses indicate that all three cultivars fell within acceptable ranges for all characteristics. Fruit Basket was judged to be more astringent, and less sweet than either Pompeii or Redskin. Of the six dwarf pepper cultivars evaluated, Pompeii was selected based upon both horticultural and organoleptic factors. Cajun Belle had the highest nutritional levels but it was judged to be too tall for space. Additionally, unlike the other candidates, Cajun Belle fruit were considerably hot when evaluated in informal taste tests and this may not be suitable for a salad crop.

IV. Conclusions

Six cultivars of tomato and six cultivars of pepper were grown in plant growth chambers maintained at environmental conditions close to what might be expected on the International Space Station. Plants were grown to an age sufficient to produce fruit. Plant sizes, yields, and nutritional attributes were measured and used to down-select to three cultivars for each species. The three cultivars for each species were then grown again and the harvested fruit underwent sensory evaluation. The combined data were compared and given weighting factors to rank the cultivars as potential candidates for testing in space. The weightings were somewhat arbitrary and gave maximum importance to plant size (smaller being good) and fruit yield (greater yields being good). For tomato, the ranking was 1) cv. Mohamed, 2) cv. Red Robin, and 3) cv. Sweet N' Neat. For pepper, the ranking was 1) cv. Pompeii, 2) cv. Red Skin, and 3) cv. Fruit

Basket. Clearly these are somewhat subjective but provide a starting point for conducting higher fidelity testing with these crops. Further testing is planned to compare these top performing cultivars under more spaceflight relevant conditions, such as the LED spectral combinations similar to the Veggie plant unit, and the use of 3000 ppm CO_2 , which is close to the current CO_2 level on the ISS. Ultimately, testing these tomato and pepper cultivars in an actual flight experiment is needed to determine how they truly perform and a spaceflight environment.

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