Effects of Supplemental Far-Red Light on Leafy Green Crops for Space

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The use of plants to provide food and eventual bioregenerative life support has been studied for nearly 50 years. A logical starting point for early missions like the International Space Station (ISS) is to grow leafy greens to supplement the crew's diet of packaged foods. In an attempt to expand the list of potential crops, NASA conducted ground studies with eight leafy greens: 'Dragoon' lettuce, 'Extra Dwarf' pak choi, shungiku, 'Barese' Swiss chard, 'Red Russian' kale, 'Toscano' kale, 'Amara' mustard, and 'Outredgeous' lettuce, which has been used in prior ground and flight tests with the Veggie Plant Chamber. Plants were grown for 28 days under 320 µmol m^{-2} s^{-1} PPFD from LED lights, 3000 ppm CO_2, and 23°C to simulate an environment similar to the Veggie Plant Chamber aboard ISS. Half of the plants were given ~7 µmol m^{-2} s^{-1} and the other half, ~23 µmol m^{-2} s^{-1} of supplemental far-red (735 nm). Supplemental far-red light resulted in increased fresh mass yields for some species but not all. This could be due to the relative small amount of far-red photons even in the supplemental treatment. 'Extra Dwarf' pak choi and 'Dragoon' lettuce produced the highest yields (70-80 g FM/plant) under both lighting regimes. A more consistent response to supplemental far-red light was increased plant canopy cover and increased shoot heights, which may be a consideration for volume constrained systems in space.

Nomenclature

ISS	=	International Space Station
LEDs	=	Light Emitting Diodes
FR	=	Far-Red
APH	=	Advanced Plant Habitat
ISS	=	International Space Station
CRL	=	Crop Readiness Level
RH	=	Relative Humidity
DI	=	Deionized Water
PPFD	=	Photosynthetic photon flux density
DAP	=	Days after planting
ANOVA	=	Analysis of Variance

I. Introduction

ONE of the current goals of space crop food production is to supplement the astronaut diet with "pick-and-eat" crops that will replenish vitamins that have a tendency degrade over time in pre-packaged, thermo-stabilized meals[1]. As space exploration efforts advance, the capability to grow supplemental fresh crops is one solution to the challenge of sustaining astronauts on long-duration missions to Mars and beyond. Moreover, determining the optimal horticultural practices to produce these crops, in the limited space available, is of utmost importance. Using light emitting diodes (LEDs), we are able to build on prior published research[2,3,4,5], and then customize light recipes to alter

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plant size and nutrient content for some leafy green species that we were testing for use in space[6]. The far-red (FR) region (700-780 nm) of the light spectrum has been studied extensively for its ability to modify growth and morphology[7,8,9,10] in plants through the actions of phytochrome.[11,12] The addition of FR radiation has been reported to affect stem and internode elongation[13,14], flowering[15,16], nutrient content[17] and other physiological responses and is reviewed by Smith et. al 1995.

When tuning the light spectrum to manipulate plant growth, the potential increase of plant canopy size and height with the addition of FR should be considered[12]. The Veggie and the Advanced Plant Habitat (APH) growth chamber systems, both currently onboard the International Space Station (ISS)[4], provide limited canopy heights of 47 cm (total) and 45 cm, respectively. While Veggie lacks FR LEDs, APH has 730 nm FR LEDs capable of a maximum output of 50 μmol^{-1} m^{-2} s^{-1}. Considering that APH offers FR LEDs with the lower amount of canopy space, it is important to understand the impacts of FR lighting regimes on leafy crops that are strategically-chosen for the space available. For these studies, we wanted to: 1) study the growth and performance of several different leafy crops under controlled environment conditions similar to what might be encountered in a space cabin environment, and 2) assess whether adding supplemental FR light could improve yields without causing excessive stem growth. These findings could help us advance these new species through a "Crop Readiness Level" (CRL)[18] evaluation for eventual spaceflight applications. In addition to growth under space relevant conditions (i.e., environmental compatibility), we have suggested that CRL assessment also include food safety considerations, nutritional analysis, and organoleptic/sensory analysis.

II. Materials and Methods

A. Growth Chamber Conditions

Leafy crops were grown in two environmental growth chambers (Percival PGW-48, 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 CO_2 levels were maintained at 23 ± 0.4 °C, 50 ± 3%, and 3000 ± 60 ppm, respectively, for all tests. These set points were chosen to simulate the environment similar to what exists in the ISS cabin air.

B. Cultural Conditions

Plant species tested included, 'Dragoon' lettuce (*Lactuca sativa*), 'Extra Dwarf' pak choi (*Brassica rapa* var. Chinesis), shungiku (*Glebionis coronaria*), 'Barese' Swiss chard (*Beta vulgaris*), 'Red Russian' kale (*Brassica napus*), 'Toscano' kale (*Brassica oleracea*), 'Amara' mustard (*Brassica carinata*), and 'Outredgeous' lettuce (*Lactuca sativa)*. Seeds were sown in 12 square plastic pots (9 cm tall, 10.2 cm width) containing 70% professional growing mix (Sun Gro, Agawam, MA), and 30% arcillite (Turface MVP, PROFILE Products LLC; Buffalo Grove, IL). In each pot, four seeds were sown on the surface of moistened media. Pots were automatically irrigated to excess several times daily with Peter's 13-2-13 (Everris, Geldermalsen, The Netherlands) nutrient solution, 1200 $\mu S\ cm^{-1}$ electrical conductivity. Trays were covered with transparent plastic and misted with deionized (DI) water for the first 3 days to promote germination. At 14 and 21 days after planting, plant pots were randomly rotated to minimize position effects.

C. Light Treatments

Two light treatments were used for the study 1) red, green, blue, UVA ("Control") and 2) red, green, blue, UVA, plus far-red ("+ FR"). Lighting was provided by Heliospectra LED grow lights (RX30, Göteborg, Sweden). Table 1 summarizes the average output of both treatments thought the experiment. The photosynthetic photon flux density (PPFD) target was 320 $\mu mol\ m^{-2}\ s^{-1}$ per treatment averaged over the entire canopy. Figure 1 shows the spectral distribution scans of all treatments taken from 350 to 800 nm at 1 nm steps with a spectroradiometer (Model PS-100, Apogee Instruments, Logan, UT). Note that there was a small amount of FR near 700 nm in the tail of the red LEDs of the control treatment, while the bulk of the FR in the "+FR" treatment centered near 730 nm (Fig. 1). The photoperiod was 16 h light/8 h dark in both treatments. Both light treatments were repeated three times, but the growth chambers were switched, to minimize potential chamber effects.

Table 1. Light recipe and average spectral data for light treatments throughout the experiment.

Parameter	Treatment	
Photon flux (μmolm^{-2} s^{-1})/light recipe ratio (%)	Control	+ FR
Average PPFD (400–700 nm)	319.6 ± 17.1	319.1 ± 20.5
UV-A (380 nm)	4.6 ± 0.5	5.0 ± 0.2
Blue (400–500 nm)	69 (23%)	69 (23%)
Green (500–600 nm)	81 (27%)	81 (27%)
Red (600–700 nm)	150 (50%)	150 (50%)
Far-red (700–800 nm)	7.6 ± 0.2	23.4 ± 0.4

D. Plant Growth Measurements

At 7 days after planting (DAP), three plants were harvested from each pot and discarded. This initial thinning removed the weakest or unsuccessfully germinated seedlings. Subsequent harvests took place on a weekly interval at 14, 21 and 28 DAP where plant growth measurements were performed. These measurements included shoot fresh and dry mass, shoot height and diameter. Additionally, internode number and internodal distance data were collected only on species where applicable. Additional measurements at 28 DAP included relative chlorophyll and anthocyanin estimates. Relative chlorophyll estimates were made using a SPAD-502DL meter (Konica Minolta Sensing, Osaka, Japan) and anthocyanin levels were estimated using a ACM-200 Plus meter (Opti-Sciences, Inc., Hudson, NH).

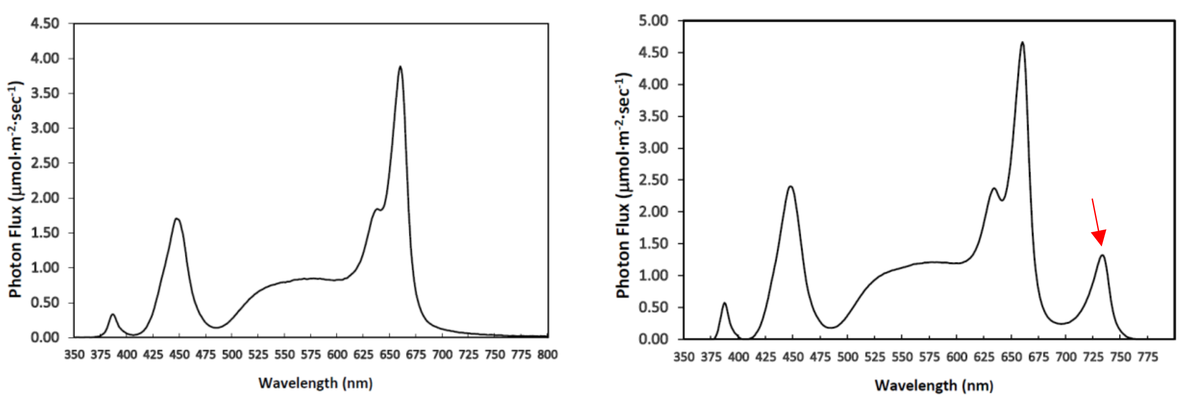

Figure 1. Spectral distribution of Control (left) and +FR (right) light treatments. Arrow indicates the peak of supplemental FR light.

E. Statistical Analysis

The experiment consisted of three replications of 28 day crop cycles. During each cycle, 96 plants (n=6 plants per cultivar × 2 treatments × 8 cultivars) were harvested on 28 DAP, making a total statistical population of 288 plants. Statistical analyses were performed using GraphPad Prism (Version 8.0.0, GraphPad Software, La Jolla, CA). Significance at the 0.05 level of significance was conducted with a one-way analysis of variance (ANOVA) followed by Tukey's multiple comparisons test.

III. Results and Discussion

A. Effects of supplemental far red on plant growth and morphology

At 28 DAP, 'Dragoon' lettuce and 'Toscano' kale showed significant increases in fresh mass, with the addition of supplemental FR radiation (Fig. 3) The effect of supplemental FR for the other species was not significant. Supplemental FR also increased the shoot height in 'Red Russian' kale, 'Toscano' kale, 'Amara' mustard, and Shungiku (Fig. 3). The other species showed no significant differences in height, shoot diameter, or number of nodes. However, intermodal distance increased significantly with supplemental FR in 'Red Russian' kale, 'Toscano' kale and 'Amara' mustard (Fig. 3).

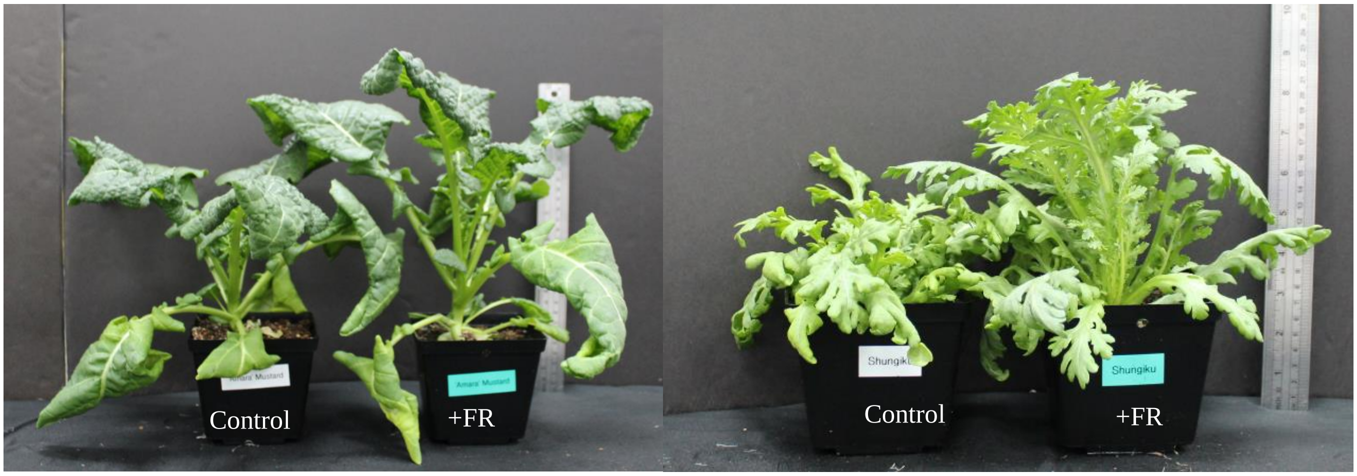

Figure 2. Increased shoot height with the addition of FR on 'Amara' mustard (left) and shungiku (right).

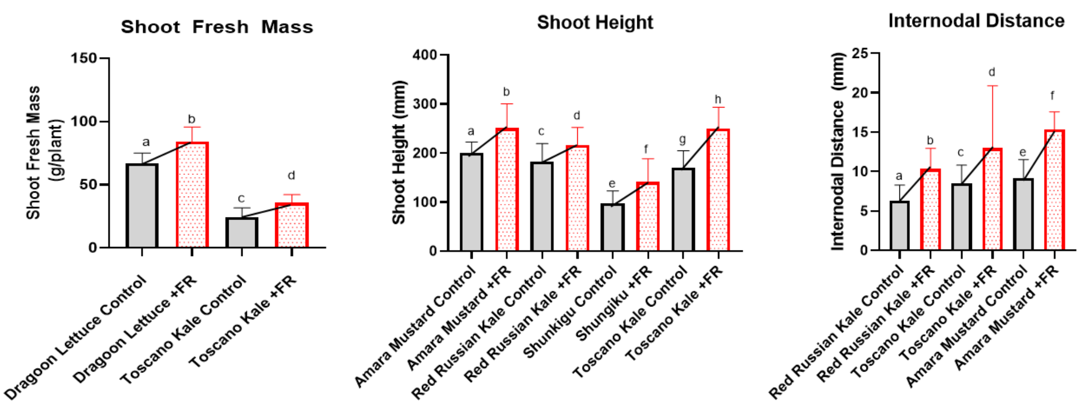

Figure 3. Fresh mass, shoot height and internodal distance of tested cultivars under Control and +FR light treatments. Note: Black lines connect Control treatment to +FR treatment within a cultivar. Within a species, means with difference letter are significantly different at the 0.05 level by Tukey's multiple comparisons test.

Chlorophyll is the green pigment found in the chloroplasts of plants. Chlorophyll measurements can be used to quantify and assess subtle changes in plant health, and are often estimated using a SPAD meter. The addition of FR supplemental FR radiation resulted in significant reduction of SPAD levels (relative chlorophyll content) in only ‘Red Russian’ kale. Chlorophyll content in all other species showed no change in SPAD values in response to supplemental FR.

Anthocyanins are a group of red / purple plant pigments that have been shown to accumulate in response to certain stressors or environmental stimuli[19,20] including different wavelengths of light. These anthocyanins can act as antioxidants in human diets and hence could offer nutritional benefits.[21,22] Estimates of leaf anthocyanin levels using a portable meter showed that supplemental FR radiation reduced the levels in ‘Outredgeous’ lettuce, ‘Extra Dwarf’ pak choi and ‘Toscano’ kale (Fig. 4). Anthocyanin estimates in all other species showed no significant difference.

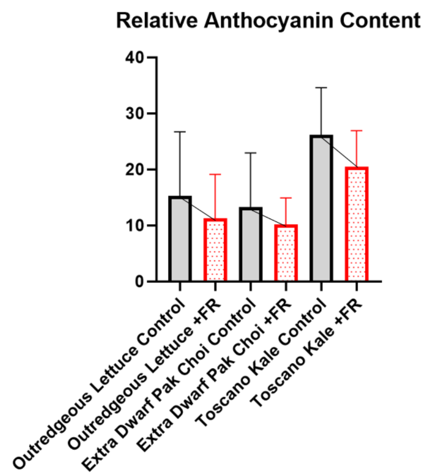

Figure 4. Relative anthocyanin content. Note: Black lines connect control treatment to +FR treatment within a species.

Table 2. Influence of supplemental FR radiation on shoot fresh weight (FW) and shoot dry weight (DW) of eight leafy green species at 28 DAP. Values with different letters are significantly different at the 0.05 level of significance by Tukey's multiple comparisons test.

	Light Treatment	'Extra Dwarf' Pak Choi	'Dragoon' Lettuce	'Amara' Mustard	'Outredgeous' Lettuce	'Red Russian' Kale	'Barese' Swiss Chard	Shungiku	'Toscano' Kale
Avg. Fresh Wt. (g/plant)	Control	74.68 a	67.12 a	64.28 a	55.69 a	54.95 a	42.36 a	34.70 a	24.45 a
	+FR	80.71 a	83.94 b	60.26 a	61.04 a	57.43 a	48.35 a	39.99 a	35.79 b
Avg. Dry Wt. (g/plant)	Control	3.30 a	2.45 a	3.03 a	2.33 a	3.67 a	2.11 a	1.76 a	2.22 a
	+FR	3.90 a	2.82 a	4.06 b	2.45 a	4.17a	2.19 a	2.10 a	3.55 b

Sequential harvest data showed supplemental FR radiation increased shoot dry mass in ‘Toscano’ kale and ‘Amara’ mustard at 28 DAP (Fig. 5; see also Table 2). Light treatment showed no significant effect for shoot dry mass for the other species across the time course harvests (Figure 5).

Our findings suggest that all of the candidate crops grew well in the controlled environment conditions (temperature, RH, and CO_2) typically encountered in space cabin environments, with some yielding better than others. Seeing differences in biomass production among species is not surprising, and might be attributed to a range of factors, including differing responses to the environmental conditions chosen, differing canopy morphologies / architectures and hence ability for radiation capture, as well as different genetic capacities for photosynthesis and carbon partitioning. Ultimately, the choices for which crops to select should be based on a range of criteria, including ease of growth, biomass yields, organoleptic acceptability, nutritional value, and food safety[18]. Based on biomass yields, 'Extra Dwarf" pak choi, 'Dragoon' lettuce and 'Amara' mustard did the best in these studies (Table 2).

The addition of FR radiation to the spectrum caused an increase yield for 'Dragoon' lettuce and 'Toscano' kale, and in shoot height and intermodal elongation of 'Amara' mustard, 'Red Russian' kale, and 'Toscano' kale. The increased yields could have been due to increased canopy cover and more radiation capture during growth[23,24], while the increased shoot height and internode length is a classic response to FR enriched environments[12]. Hence you could make a case for supplementing with FR for some species based on yield, but not for others, based on their taller, less volume efficient morphology. It is interesting to note that the "head" forming species such as lettuce and the pak choi showed no response to supplement FR with regard to shoot height. Our choice to supplement with just ~20 µmol m^{-2} s^{-1} of FR was somewhat arbitrary, and increasing the level of FR might have amplified the effects, and should be researched further. But our findings suggest that a relative small amount of FR light added to a light spectrum can have pronounced effects on some crops, which is consistent with the volumes of literature from phytochrome and spectral quality studies over the years[12] .

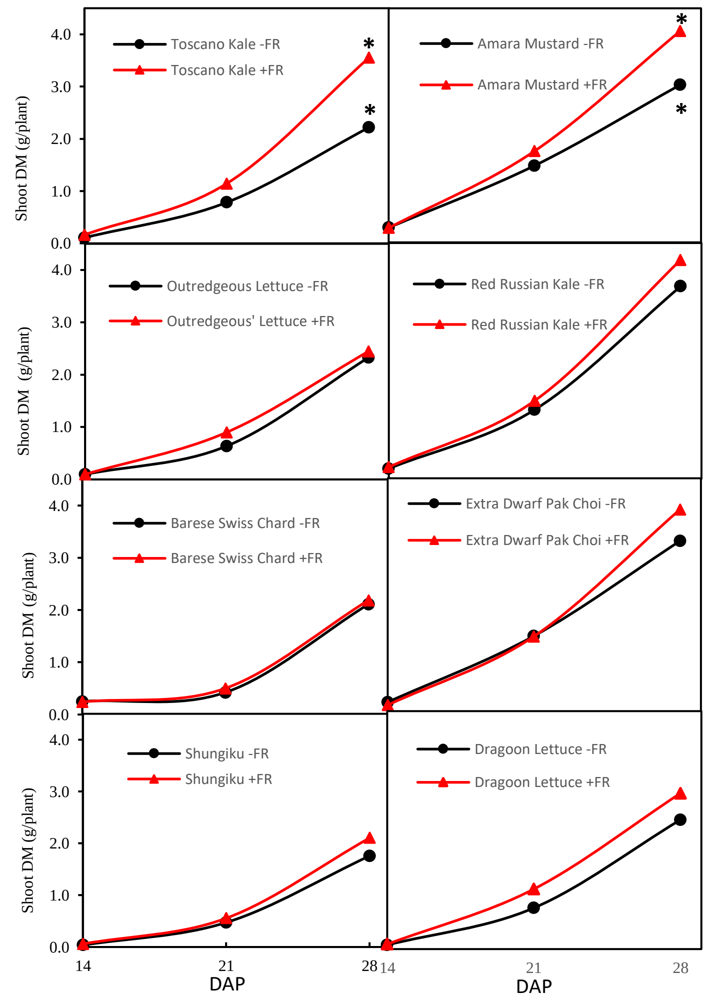

Figure 5. Total dry mass (DM) accumulated over time for both light treatments for all eight cultivars. Time points with asterisk are significantly different at the 0.05 level if significance by Tukey's multiple comparisons test**.**

In addition to phytochrome effects on stem elongation and leaf expansion, which would affect radiation capture, recent studies suggest that FR photons can be photosynthetic.[25] This is likely due to a synergistic effect between FR and short wavelength photons, which help balance photosystems I and II and improve overall photosynthetic efficiency.[25] The beneficial effects of longer red and FR radiation on photosynthesis were pointed out by Emerson et al[26] and others (see Zhen and Bugbee[25]) and now suggest that the role of FR in plant growth is multifaceted—affecting not just plant morphology but also photosynthetic efficiency. Based on this, further studies using even higher levels of supplemental FR with these same species could be revealing and important to understand for future applications with plants for food production and life support in space.

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