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ORIGINAL PAPERS

CAVITY RING DOWN SPECTROSCOPY AS A TOOL FOR MONOCHROMATIC LIGHT ACTION ON TOMATO PLANTS IN BIO-REGENERATIVE LIFE SUPPORT SYSTEMS

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Abstract. *Narrow spectrum LEDs, which regulate diverse photo-morphogenetic responses of plants, can be used to achieve desired plant responses in terms of germination, growth rate, and productivity. Current study examined the effect of blue (465-475 nm), green (515-530 nm), red (620-630 nm), and cool-white light (CCT 6000-6500K) on tomato (Solanum lycopersicum) different cultivars, with determinate and indeterminate growth. Our findings show that monochromatic light had a substantial impact on germination, growth, hydration status, and $\delta^{13}C$ values in plantlets grown under experimental conditions. When compared to the other wavelengths, red light stimulates the most and visible light inhibits the most germination in the selected tomato varieties. The lowest elongation was measured in visible light and the greatest elongation was measured in red light, resulting in a drop in the PPFD from 326.1 to 179.4 $\mu mol \cdot m^{-2} \cdot s^{-1}$. Our findings imply that the $\delta^{13}C$ signature in plants detected by Cavity Ring Down Spectroscopy could be a useful proxy for quickly assessing the physiological condition of plantlets in their early stages of development in Bio-Regenerative Life Support Systems.*

Keywords: monochromatic light, Bio-Regenerative Life Support Systems, $\delta^{13}C$, photo morphogenetic response.

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INTRODUCTION

In manned space missions, the provision of life support materials, including those necessary for sustaining human life, is an integral component either carried as part of the mission support materials or regularly supplied from Earth. The International Space Station (ISS) is exposed to challenging conditions such as high radiation levels (1.5 kW/m2), high vacuum, low gravity, and low temperatures [1]. Onboard the ISS, constraints related to limited weight, volume, and power supply exist. Consequently, the development of a life support system capable of meeting human nutritional requirements in such a hostile environment would address significant limitations in the scope of human space exploration.

Integrating higher plants into regenerative life support systems poses a considerable challenge due to unsuitable space conditions for plant growth. However, incorporating higher plants could facilitate the replenishment of fresh vegetables and the regeneration of fresh water through transpiration, creating a more psychologically friendly environment for the crew [2].

The establishment of Bio-Regenerative Life Support Systems (BLSS) necessitates controlled growth chambers with precise regulation of nutrient supply, photosynthetic active radiation (PAR), temperature, humidity, radiation shielding, atmospheric composition, and pressure. Achieving optimal productivity under controlled environmental conditions is the primary goal of controlled environmental plant production. Currently, a lighting system meeting the stringent requirements of space-based controlled environments, which is actively cooled and highly integrated with other subsystems, is not available.

Desired plant responses, such as growth rate and productivity, may be achieved using narrow spectrum LEDs. The quality of light plays a crucial role in regulating various photo-morphogenetic responses of plants, including seed germination, stem elongation, leaf expansion, flowering, senescence, and stress reactions against biotic and abiotic factors [3-6].

The growth of plant organs relies on carbohydrates produced through photosynthesis under specific light conditions. Different developmental stages require varying levels of carbohydrates, and any deficits may lead to the suppression of fruit development and other morphological disorders [7]. When the daily PAR is limited in the available experimental facility, adjusting the low carbohydrate production rate to align with a low development rate may be achieved by lowering the temperature in the growth chambers [8.9].

Temperature is a critical factor in space conditions' growth chambers due to the suppression of heat convection at lowered gravity levels, leading to increased leaf temperature surface and modified evapotranspiration [10]. Altered water status directly affects plant growth, development, and productivity. Therefore, proper air movement is essential to enable normal transpiration rates and water transport in higher plants growing in space [11,12].

Biomass production is closely linked to plant water status, and two indices, effective use of water (EUW) and water-use efficiency (WUE), have been developed as determinants of yield under stress. EUW focuses on maximal moisture capture and reduced non-stomatal transpiration, while WUE involves water uptake followed by subsequent evapotranspiration. Improving EUW is a major target for enhancing yield in water-limited environments, as it sustains assimilate partitions and reproductive success [13,14]. WUE is commonly measured at the leaf level, though such measurements may not align with daily integrals or whole plant estimates of WUE [15].

Cavity Ring-Down Spectroscopy has been employed as a tool for characterizing evapotranspiration partitioning of water isotopes [16,17] or monitoring ethylene levels [18].

The first objective of this study was to determine whether Cavity Ring Down Spectroscopy (CRDS) accurately estimates growth during specific developmental phases and plant productivity in a BLSS. The second objective was to assess the correlation between different stages of development and δ13C values of three tomato cultivars (Heinz, Ghita, and Elisabeta) grown under different light-emitting diodes: blue (465-475 nm), green (515-530 nm), red (620-630 nm), and cool-white light (CCT 6000-6500K).

MATERIALS AND METHODS

The investigation encompassed three distinct cultivars of tomato (*Solanum lycopersicum* L.): one exhibiting determinate growth (Heinz), and two demonstrating indeterminate growth (Elisabeta and Ghita – CS 45 48). Disparities in growth patterns, nutritional requisites, disease resilience, and fruit characteristics between determinate and indeterminate tomato cultivars are notable. Cultivars with determinate growth manifest diminished stature with constricted branching, rendering them more conducive to restricted growth environments such as those encountered aboard the International Space Station (ISS). Determinate plants exhibit a more consolidated early fruit harvest, whereas indeterminate varieties yield more evenly across an extended harvesting period.

The experimental design followed a complete block arrangement. Two hundred seeds of each cultivar were sown onto two layers of filter paper within Petri dishes and irrigated with 20 mL of sterile water. Germination assessments comprised four replications of 50 seeds per cultivar. Parafilm was utilized to seal the Petri dishes to prevent desiccation, subsequently placing them within germination chambers equipped with diverse light-emitting diodes (LEDs) (Sartorius Certomat BS-1, Goettingen, Germany). LEDs emitting light across varying wavelengths - blue (465-475 nm), green (515-530 nm), red (620-630 nm), and cool-white light (CCT 6000-6500K) (Arelux, Romania) - uniformly illuminated the chambers. Petri dishes were randomly positioned within the growth chambers and periodically rotated. Germination, defined as radicle emergence surpassing 1 mm, was recorded at 7 days. Seedlings were subsequently

transplanted into other Petri dishes and sustained for 21 days. All manipulations were conducted under dim green light conditions (553 ± 8 nm, <0.2 μmol·m^{-2}·s^{-1}) according to other papers [19]. Biometric assessments of root and above-ground lengths were conducted on days 6, 10, and 21.

Subsequently, plants were cultivated hydroponically for 60 days using a modified Cooper's solution containing (mg·L^{-1}) N 118, P 30, K 150, Ca 92.5, Mg 25, S 34, Fe 6, Mn 1.0, Zn 0.1, Cu 0.1, B 0.15, Mo 0.2. pH was maintained at 5.8 throughout the experiment. Initially, 50 mL of nutrient solution per plant was administered for the first 4 weeks, followed by 120 mL per plant for the subsequent 4 weeks. On the 45th and 60th days, $\delta^{13}C$ content was assessed.

The growth conditions were carried out in controlled environment in adapted growth chambers (Sanyo MLR-351, Osaka Japan) with a 12/12 h light cycle and 65% humidity with a temperature of 25±0.1ºC and 20±0.1ºC (day/night), and photosynthetic photon flux density (PPFD) according to the Table 1.

Table 1. Light characteristics of plantlets exposed to a 12 h daily light phase

Light (nm)	**PPFD μmol·m^{-2}·s^{-1}**	**PAR mol·m^{-2}·d^{-1}**
Blue (465-475)	297.6	12.88
Green (515-530)	237.6	10.28
Red (620-630)	179.4	5.57
White (400-700)	326.1	14.09

For biomass determination, ten plants per cultivar were harvested initially, after 45 days, and at the termination of the experiment. Above-ground and root components were segregated and desiccated at 60°C to ascertain dry weight. Total biomass increment was calculated as the disparity between whole-plant dry weights at the experiment's onset and conclusion. Plant water status over the 45- and 60-day intervals was estimated via the disparity between dry and fresh weights.

Carbon isotope composition analysis involved sampling leaves and stems per cultivar and treatment from distinct plants. Samples were dried, milled, and homogenized before analysis. Isotopic $\delta^{13}C$ values were determined using a Picarro G2121-i type analyzer (Cavity Ring Down Spectroscopy) [20], coupled with a combustion module (Costech Analytical Technologies Inc.) at the Stable Isotope Laboratory of Babeş-Bolyai University in Cluj-Napoca, Romania. Details regarding sample analysis are outlined elsewhere [21,22]. $\delta^{13}C$ values were expressed relative to VPDB [23] employing the equation:

$$\delta^{13}C = [(R_{sample}/R_{standard})-1] \times 1000 \ (‰)$$

where R represents the $^{13}C/^{12}C$ ratio. Each sample underwent two to four measurements, and the mean was reported.

The precision was within ±0.08‰ (1σ) based on measured samples (ranging between 0.04 and 0.08‰, with an average of 0.06‰) and was contingent upon sample homogeneity. For direct measurements on leaf and stem, the precision was within ±0.47‰ (1σ), signifying the variability of $\delta^{13}C$ values among different anatomical segments rather than diminished precision. The reproducibility of measurements, verified via replicate standard measurements (standard B2155 from Elemental Microanalysis Ltd.) in each run, was within ±0.14‰ (1σ) on average ±0.1‰.

Statistical analysis of experimental data employed ANOVA. Significance of treatment effects was determined by assessing the magnitude of the F-value ($P \leq 0.05$). When a significant F-test was observed, means were separated using Fisher's test [24,25]. The null hypothesis posited uniformity among population means of plants cultivated under distinct light conditions, with the alternative hypothesis suggesting at least one population mean differs.

RESULTS AND DISCUSSIONS

The present study employed light with Photosynthetic Photon Flux Density (PPFD) ranging from 179.4 to 326.1 $\mu mol \cdot m^{-2} \cdot s^{-1}$, corresponding to a Photosynthetically Active Radiation (PAR) within the interval of 5.57 to 14.0 $mol \cdot m^{-2} \cdot d^{-1}$, contingent upon various LED wavelengths. These parameters were selected based on prior research, which revealed that PPFD levels below 150 $\mu mol \cdot m^{-2} \cdot s^{-1}$ led to the development of tomato plants characterized by thin leaves and diminished dry matter content. Conversely, it has been documented that higher PAR exceeding 20 $mol \cdot m^{-2} \cdot d^{-1}$ (e.g., 16 hours exposure at 350 $\mu mol \cdot m^{-2} \cdot s^{-1}$) cannot be effectively utilized by young tomato plants in the exponential growth phase [26].

Table 2. Percentage (%) of germination of tomato cultivars at different wavelengths

Cultivars	Wavelengths (nm)			
	400-700	465-475	515-530	620-630
Heinz	50	55	70	95
Ghita	40	45	60	60
Elisabeta	45	75	55	50
Mean	45	58.33	61.67	68.33

The findings pertaining to seed germination, as summarized in Table 2, reveal discernible distinctions among the three tomato cultivars under investigation. Specifically, the Heinz cultivar exhibited heightened sensitivity to red light, achieving a germination rate of 95%, whereas the Elisabeta cultivar demonstrated greater responsiveness to blue light, resulting in a germination rate of 75%. On average, red light elicited the most pronounced stimulation, while the visible spectrum exerted the greatest inhibitory effect on germination compared to other wavelengths. Previous

studies have identified the efficient stimulation of germination in seeds sensitive to red spectral range light (~660 nm) [27]. Hourly applications of red pulses have been shown to partially replicate the effects of continuous red irradiation on seed germination in tomatoes, indicating the existence of two distinct responses: an inducing reaction triggered by repeated red pulses and a non-inducing response specifically facilitated by continuous red light [28]. The effects of blue light on germination vary depending on the species and irradiation protocol, with inhibition of germination occurring through different mechanisms.

Plants possess two distinct physiological systems for light perception, spanning a broader range of wavelengths and photon fluence. Phytochromes (Phy), serving as photoreceptors involved in photo-morphogenetic responses, play a crucial role in regulating the expression of numerous light-responsive genes [29]. PhyA triggers the irreversible photoinduction of seed germination upon exposure to extremely low light fluence in the UV-A, visible, and far-red range, while PhyB mediates the photo-reversible response, primarily activated by red and far-red light at significantly higher fluences than those required for PhyA activation [30]. Specific light wavelengths transduced by PhyB modulate the expression of various genes associated with hormone biosynthesis and signaling. A complex interplay between light and temperature signals, partly mediated by hormones such as gibberellin (GA), abscisic acid (ABA), and jasmonic acid (JA), governs the processes of germination and plant growth [31].

Table 3. Growth of tomato plantlets under different light irradiation (cm)

Time	Cultivars	Wavelengths (nm)			
		400-700	**465-475**	**515-530**	**620-630**
6 days	Heinz	1.44±0.55	1.57±0.28	2.00±0.62	2.78±0.56
	Ghita	1.54±0.49	1.86±0.50	1.95±0.67	2.73±0.67
	Elisabeta	2.25±0.52	2.16±0.35	2.89±0.50	2.90±0.76
10 days	Heinz	2.20±0.37	2.27±0.50	3.11±1.00	5.78±1.35
	Ghita	2.24±0.35	2.45±0.41	3.41±1.03	6.08±1.17
	Elisabeta	2.39±0.48	2.66±0.44	2.97±0.61	6.44±0.83
21 days	Heinz	3.14±0.61	2.89±0.59	4.72±0.73	6.75±1.81
	Ghita	3.48±0.45	4.01±0.53	4.94±0.96	6.67±1.69
	Elisabeta	2.87±0.48	4.68±0.87	3.74±0.72	8.12±1.18

Regarding the growth of tomato seedlings under different wavelengths and photosynthetic photon flux density (PPFD), as outlined in Table 3, it is evident that light quality induces distinct developmental patterns in plant seedlings. Signals perceived by photoreceptors activate downstream regulators, initiating photomorphogenic developmental processes such as root and shoot elongation. Numerous studies have elucidated the antagonistic effects of light and darkness on hypocotyl elongation, mediated by external and internal cues including plant photoreceptors, phytohormones, calcium, and transcription factors. However, the

precise coordination of these downstream regulators to mediate differential hypocotyl growth responses to light remains largely unresolved [32].

In this study, we sought to ascertain the optimal duration for eliciting specific wavelength-induced growth responses in plant seedlings, even within the initial six days post-germination, significant differences in seedling growth were observed. The Heinz cultivar exposed to blue light (465-475nm) exhibited the least elongation, while the Elisabeta cultivar under red light (620-630nm) demonstrated the greatest growth. These disparities persisted throughout the 21-day observation period. Generally, the lowest elongation occurred under visible light, with the highest elongation observed under red light, corresponding to a decrease in PPFD from 326.1 to 179.4 $\mu mol \cdot m^{-2} \cdot s^{-1}$. Plantlets grown under low PPFD levels, not exceeding 200–250 $\mu mol \cdot m^{-2} \cdot s^{-1}$, exhibited a significantly reduced growth rate, regardless of the light source, complicating the elucidation of other lighting parameters' effects on plant growth [3]. Notably, at a PPFD of 179.4 $\mu mol \cdot m^{-2} \cdot s^{-1}$, plant elongation under red light was markedly higher (282.9%) than under visible light for the Elisabeta cultivar and 214.9.9% higher for the Heinz cultivar. The sensitivity of the indeterminate growth cultivar to red light was 280% higher compared to the determinate growth cultivar. After 21 days, the difference in elongation between determinate and indeterminate growth cultivars was merely 20.29%. Given the limited space within growth chambers and the potential for inducing elongation in both cultivars, the Heinz cultivar, exhibiting determinate growth, appears to be the preferable option for Bioregenerative Life Support Systems (BLSS). Similar results regarding the effects of red light on elongation have been reported in previous studies utilizing various plant species, notably lettuce [33].

Table 4. Percentages of DW and water from FW (%)

		45 days				**60 days**			
		Roots		**Shoots**		**Roots**		**Shoots**	
Wavelength (nm)		**DW**	**Water**	**DW**	**Water**	**DW**	**water**	**DW**	**water**
400-700	Heinz	6.7	93.3	6.0	94.0	8.8	91.2	6.1	93.9
	Ghita	1.77	98.2	4.8	95.2	5.8	94.2	5.4	94.6
	Elisa	5.3	94.7	3.7	96.3	5.5	94.5	7.3	92.7
465-475	Heinz	5.5	94.5	6.0	94.0	8.7	91.3	6.8	93.2
	Ghita	5.7	94.3	5.8	94.2	7.6	92.4	6.2	93.8
	Elisa	5.9	94.1	5.8	94.2	5.6	94.4	8.2	91.8
515-530	Heinz	8.1	91.9	6.1	93.9	8.2	91.8	7.4	92.6
	Ghita	7.6	92.6	5.5	94.5	8.1	91.9	6.3	93.7
	Elisa	7.0	93.0	6.3	93.7	7.4	92.6	7.5	92.5
620-630	Heinz	4.1	95.9	5.8	94.2	4.3	95.7	6.3	93.7
	Ghita	4.8	95.2	4.7	95.3	6.7	93.3	4.3	95.7
	Elisa	4.8	95.2	4.9	95.1	5.1	94.9	3.7	96.3

Regarding the potential to induce desired plant responses by modulating wavelengths and PPFD, the Heinz cultivar exhibited the highest sensitivity to light treatment (193.05% difference between the highest and lowest effects) compared to the other two cultivars within the first six days post-germination. Under visible light, the Elisabeta cultivar maintained a low elongation rate throughout the 21-day observation period, while seedlings exposed to red light exhibited etiolation characterized by a short primary root, rapidly elongating hypocotyls, and small unopened cotyledons.

Dry weight percentage and water content analyses, presented in Table 4, revealed noteworthy findings. At 45 days, monochromatic green light irradiation resulted in the highest dry weight percentage across all light conditions for all cultivars, particularly at the root level. Significant increases in root dry weight were observed under visible light between days 45 and 60 (+4.03%) for the Ghita cultivar, and in shoot dry weight (+3.6%) for the Elisabeta cultivar. The highest water content was recorded under red light.

The measurements of $\delta^{13}C$ values of plant samples indicate a significant stable carbon isotope fractionation induced by different light conditions, the effect varying among the different varieties. The results are presented in Fig. 1 and Fig.2. The preliminary $\delta^{13}C$ values measured on leaf and stem of tomato plants indicate variable isotopic composition, but when the plant samples where milled and homogenized, the precision was higher than ±0.08‰ (1σ).

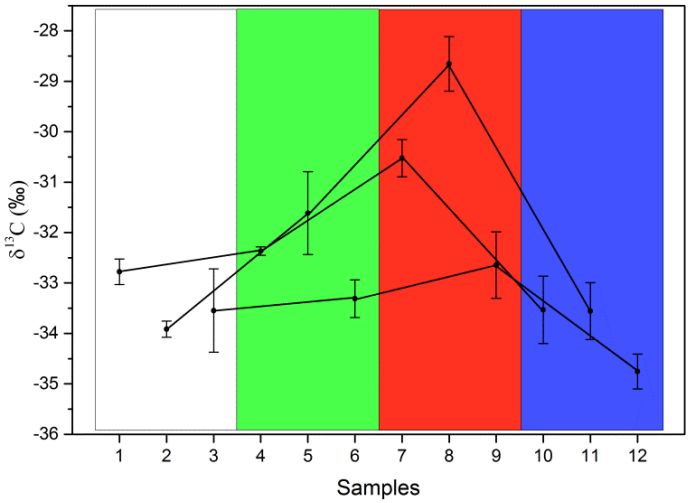

Fig.1 $\delta^{13}C$ values in plantlets grown in experimental conditions at 45 days. From left to right the colors represent white, green, red and blue light. Numbers are indicating cultivars: 1,4,7,10-Heinz, 2,5,8,11-Ghita, 3,6,9,12-Elisabeta

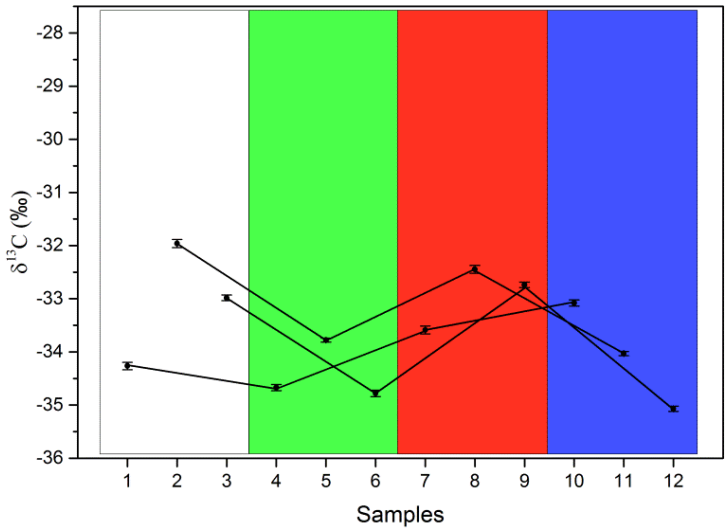

Fig.2 $\delta^{13}C$ values in plantlets grown in experimental conditions at 60 days. From left to right the colors represent white, green, red and blue light. Numbers are indicating cultivars: ,4,7,10-Heinz, 2,5,8,11-Ghita, 3,6,9,12-Elisabeta

Isotope analysis is a well-established tool used to determine carbon fixation pathways of plant species and to study their physiological processes. Plants contain less ^{13}C than the atmospheric CO_2 which is used in photosynthesis. They are therefore "depleted" of ^{13}C relative to the atmosphere. This depletion is caused by enzymatic and physical processes that discriminate against ^{13}C in favor of ^{12}C. Different isotopic signatures based on photosynthetic pathways, and on eco-physiological features have been identified. The diffusion of CO_2 from the atmosphere through boundary layer, stomata, mesophyll to chloroplast has an apparent fractionation ($\Delta\delta$) of ~4.4‰ due to the slower motion of the heavier ^{13}C containing CO_2 molecules, followed by the discrimination of Rubisco against the ^{13}C, with a $\Delta\delta$ of about 29‰. $\Delta^{13}C$ values for C3 plants lie between −25 to −35‰, with a median of about −27‰. Stable carbon isotope composition varies in plant tissues due to differences among their chemical components: lipids are 10‰ lighter whereas cellulose and other carbohydrates are typically 1–2‰ heavier than whole tissue [34].

In this experiment, $\delta^{13}C$ values vary from –28.65 to –35.7‰. Applying Fisher test to compare the influence of monochromatic light on $\delta^{13}C$ values at 45 days, significant differences were recorded between red and white ($p = 0.015$) and between and blue and red light ($p = 0.008$), in red light existing the highest value of $\delta^{13}C$ stable isotope. The differences are not significant after 60 days of the experiment, suggesting an initial sensitivity of plantlets to certain light characteristics.

The production and maintenance of biomass primarily rely on carbohydrates synthesized through photosynthesis, influencing carbon partitioning between source

and sink tissues, thereby impacting plant growth and development. Manipulation of carbon allocation between roots and shoots via specific light wavelengths may stimulate water and mineral uptake or photosynthesis [36,37]. The $\delta^{13}C$ values of plant samples, indicative of significant stable carbon isotope fractionation induced by different light conditions, varied among the different varieties.

Quantum yield, referencing values obtained in red light (600-625 nm), which effectively drive photosynthesis, may elucidate the observed significant CO_2 fractionation induced by red light (620-630nm). The relatively high isotopic discrimination observed may be attributed to red light's influence on stomatal conductance, photosynthetic efficiency, and plant water status. Carbon isotope ratios serve as proxies for photosynthetic water-use efficiency (WUE), further enhancing our understanding of physiological processes in plantlets within Bioregenerative Life Support Systems (BLSS) [34].

This study provides valuable insights into the specific effects of narrow-spectrum LEDs on many elements of tomato plant growth and development. The discoveries advance our understanding of how light spectra might be adjusted to accomplish desired results in controlled conditions.

Continuous developments in LED technology may have happened in recent years, including increases in energy efficiency, spectrum tailoring, and the production of LEDs with novel wavelengths that target specific plant responses. The integration of smart technology, such as sensors and feedback control systems, enables real-time monitoring of plant responses, which can lead to dynamic alterations in lighting conditions to improve plant growth and resource efficiency.

Machine learning algorithms and data analytics are becoming increasingly used in controlled-environment agriculture. The integration of light requirements with other environmental elements such as temperature, humidity, and nutrient availability might aid in fine-tuning cultivation procedures for maximum plant growth. Plant health and stress can be monitored non-invasively using biological signatures like $\delta^{13}C$ readings.

CONCLUSIONS

Our study underlines the significant impact of monochromatic light on germination, growth, water status, and $\delta^{13}C$ values in tomato plantlets under experimental conditions. Red light emerged as the most potent stimulant of germination, with Heinz cultivar exhibiting the highest sensitivity. The manipulation of light wavelengths and PPFD facilitated desired plant responses, with the Heinz cultivar displaying the greatest sensitivity. Our findings highlight the potential of Cavity Ring Down Spectroscopy (CRDS) as a valuable tool for assessing the physiological state of plantlets within BLSS, shedding light on carbon partitioning and yield manipulation mechanisms. Further research elucidating the regulatory

mechanisms underlying isotopic composition and its implications for plant growth is warranted to optimize plant cultivation in constrained environments.

Beyond traditional agriculture, the use of narrow spectrum LEDs and precise light management has implications for various controlled environment agriculture systems, such as vertical farming, urban agriculture, and space farming.

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