THESIS

DYNAMIC MANIPULATION OF FAR-RED LIGHT AND TEMPERATURE FOR THE PRODUCTION OF MICROGREENS IN CONTROLLED ENVIRONMENTS

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ABSTRACT

DYNAMIC MANIPULATION OF FAR-RED LIGHT AND TEMPERATURE FOR THE PRODUCTION OF MICROGREENS IN CONTROLLED ENVIRONMENTS

Microgreens are becoming increasingly popular for controlled environments due to their ease of production, profitability, and high concentration of nutrients. However, to date there is little information on how light spectra and temperature interactively affect plant growth and morphology to optimize the production of horticultural crops, including microgreens. Therefore, the objective of this study was to investigate the benefit of reducing air temperature as well as supplementing with far-red (700-750 nm) photons to enhance the morphology and phytochemical concentrations of three *Brassica* microgreen species. Seeds of mustard (*Brassica juncea* 'Garnet Giant'), kohlrabi (B*rassica oleracea* var. *gongylodes*), and red cabbage (*Brassica oleracea* var. *capitata*) were sown on rockwool substrate and grown in walk-in growth chambers using ebb and flow hydroponic systems at the CSU Spur campus. Upon germination, microgreens were grown under ambient air temperature of either 18 or 21 ºC and subjected to the following lighting treatments: photosynthetic photon flux density (PPFD) of 165 $\mu mol \cdot m^{-2} \cdot s^{-1}$ (PAR_{165}); PPFD of 200 $\mu mol \cdot m^{-2} \cdot s^{-1}$ (PAR_{200}); and PPFD of 165 $\mu mol \cdot m^{-2} \cdot s^{-1}$ + 35 $\mu mol \cdot m^{-2} \cdot s^{-1}$ of far-red light (PAR_{165}+FR_{35}). Expected shade avoidance responses (e.g., increased hypocotyl elongation) due to a low R:FR value occurred in all three species under PAR_{165}+FR_{35}, regardless of temperature. Additionally, fresh weight of red cabbage and kohlrabi was greatest under PAR_{165}+FR_{35} or similar between PAR_{165}+FR_{35} and

PAR_{200}, respectively, at both temperatures. While an interaction with temperature was not observed, results support the role of far-red light in the manipulation of both microgreen quality and biomass accumulation.

A follow-up experiment was conducted with red cabbage microgreens grown under the previous far-red lighting treatment (PAR_{165}+FR_{35}) to explore the dynamic manipulation of air temperature throughout production. While production under PAR_{165}+FR_{35} should result in characteristic shade avoidance responses, including a potential decrease in pigmentation, we hypothesized that a reduction in air temperature during production could serve as a secondary stressor to increase phytochemical concentrations (e.g., anthocyanins). Using the same experimental setup described for the previous experiment, red cabbage microgreens were grown at an air temperature of 21 ºC for 12 days (Control) or moved 6, 8, 10, or 11 days after sowing to an air temperature of 16 ºC. Shade avoidance responses (e.g., hypocotyl elongation) and both fresh and dry weight were reduced for microgreens transferred to the air temperature of 16 ºC on days 6 and 10 compared to all other treatments. Interestingly, microgreens transferred to the air temperature of 16 ºC resulted in greater dry weight compared to Control. While results were not significant, an anticipated trend of increased relative anthocyanin content in response to longer durations at the 16 ºC air temperature was observed. Although the dynamic manipulation of both far-red light and temperature could lead to microgreens with optimal yield, morphological characteristics, and phytochemical concentrations, future research is warranted to elucidate target environmental setpoints, durations, and possible interactions.

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CHAPTER 1. INTRODUCTION AND LITERATURE REVIEW

1.1 Controlled Environment Agriculture Production and Public Interest

Crop production in controlled environments is expanding globally, evoking a need for empirical testing to establish species-specific guidelines and production practices. Controlled environment agriculture (CEA) is one of many terms used to describe the monitoring, control, and automation of crop production in a protected environment (Ramin Shamshiri et al. 2018). Controlled environment agriculture provides a means by which crops can be grown year-round, regardless of location, with facilities ranging from greenhouses to indoor vertical farms (Ares et al. 2021). What often differentiates CEA facilities is their capacity for environmental control. Fully indoor operations use a variety of technologies to ensure environmental setpoints are held with limited variation, whereas a greenhouse may involve less control infrastructure, as the local environment is utilized to reduce production inputs (Ares et al. 2021).

Fully indoor operations rely solely on electric fixtures for crop lighting needs, often referred to as sole-source lighting (Park et al. 2022). Unfortunately, the use of sole-source lighting results in high electrical energy costs when compared to operations utilizing solar radiation to support plant production (e.g., greenhouses). Instead, for greenhouse production, supplemental lighting technologies are commonly used to supplement natural light with electric sources to achieve optimal crop lighting parameters (Morrow 2008). Regardless of the method, technologies that facilitate the manipulation and optimization of lighting have allowed CEA to approach production differently, such as vertically integrated systems (Ramin Shamshiri et al. 2018).

The use of both supplemental and sole-source electric lighting exposes current aspects of CEA that may be wasteful from a material and energy standpoint (Pérez-Bermúdez and Rognoni Martínez 2023). Production in controlled environments requires both an intense upfront and reoccurring cost, making improvements to technologies and practices necessary for industry advancement. In 2014, the average annual gross income of a greenhouse vegetable farm was around $700,000 per hectare (USDA 2014). Concurrently, the annual energy cost of electric lighting for growing greenhouse vegetables with a 16-h photoperiod in 2014 was around $180,000 per hectare (USDA 2014; van Iersel 2017). Reducing the cost of lighting in greenhouse settings would allow growers to maximize profits, while also improving horticultural practices. Such statistics provide reasoning as to why there has been a decline in CEA production in the United States since 2014 (Davis and Lucier 2021). USDA census data provides insight on the temporal rise and decline of CEA production in the United States. For example, greenhouse bell pepper square footage increased from 2009 to 2014 by 186%, only to drop from 2014 to 2019, to –29%, with a negative percent indicating imports needed to sustain demand (Davis and Lucier 2021). The same trend followed for cucumbers and lettuce (lettuce not to the extent of requiring imports), with tomatoes being the only greenhouse crop from 2014 to 2019 to increase production in terms of square footage (Davis and Lucier 2021).

Until energy-intensive variables are curated for crop-specific production protocols, most large-scale CEA operations will continue to fall short of their maximum potential (Ramin Shamshiri et al. 2018) or cease to operate (Davis and Lucier 2021). This paints a clear picture as to why multimodal experiments are needed to fine tune environmental inputs for CEA. To date, research has often prioritized system concepts rather than optimization of parameters for individual species (Ramin Shamshiri et al. 2018).

One area of CEA that has steadily integrated itself into the United States industry is the production of microgreens (Lensing 2018). The minimal inputs and square footage necessary for production allow for different modes of cultivation outside of traditional CEA operations including residential, culinary, and commercial settings (Moraru et al. 2022; Verlinden 2019). A major contributing factor to the increase in popularity of microgreens production is a heightened increase in health mindfulness by the public alongside the naturally high concentration of phytonutrients associated with microgreens (Kyriacou et al. 2016).

1.2 Microgreens

Microgreens are seedlings, usually vegetables, that are harvested 7 to 21 days after germination (Choe et al. 2018; Moraru et al. 2022). The temporal range is due to both variation in growth rates across species and the efficiency of production based on optimization of inputs. Such a range is also due to the neighboring stages of growth for microgreens; sprouts and baby greens. The defining characteristics between these three products of a single species are a function of both production method and the portion of the crop consumed. Sprouts are seeds germinated in the dark and consumed as a shoot. Sprouts are generally chlorotic due to a lack of light, with production requiring less than 10 days (Choe et al. 2018). Cultivation of baby greens is longer than that of microgreens, as only true leaves are consumed. As such, baby greens typically require over 21 days to achieve a harvestable product (Choe et al. 2018). Microgreens are positioned between these two stages in terms of time to harvest, with the product consumed including the hypocotyl, cotyledons, and possibly 1-2 true leaves (Choe et al. 2018; Verlinden 2019). A wide variety of species are utilized for the production of microgreens including arugula (*Eruca sativa)*, kohlrabi (*Brassica oleracea*), mizuna (*Brassica rapa* var. *nipposinica*), mustard (*Brassica juncea)*, radish (*Raphanus sativus*), red cabbage *(Brassica oleracea* var. *capitata*), and

wasabi (*Wasabia japonica*) (Choe et al. 2018; Verlinden 2019). This variety provides consumers the opportunity to experience familiar foods in an entirely new way, as species and cultivar differences lead to an assortment of colors, textures, and flavors (Xiao et al. 2012).

Microgreens are naturally nutrient dense, and require a shorter, less intensive production interval than their full-size counterparts, making them a crop of high interest and profit return (Liu et al. 2022; Weber 2017). For example, Lester et al. (2010) concluded that younger spinach leaves (*Spinacia oleracea* L.*)* have higher concentrations of phytonutrients and vitamins than their older counterparts. Given microgreens are grown for a short duration, this adds plausibility to the belief that they are nutrient dense, despite a general lack of scientific data on species-specific nutritional content (Xiao et al. 2012). The potential for nutrient dense crops is particularly of interest in developed areas of the world lacking either adequate nutrients or optimal farming climates (Weber 2017). Urban areas are often highlighted, as their need for fresh food products coincides with a generally large market to support production. Other benefits of microgreen production include a short production cycle, resulting in minimal fertilizer inputs; this short production cycle also reduces or entirely removes the need for pesticides (Weber 2017). Overall, the low-input and maintenance for microgreens production positions them as an ideal crop for CEA. This convenience follows microgreens from their production protocols through to methods of consumption.

1.3 Growing Parameters for Microgreens in a Controlled Environment

Each piece of equipment encompassing CEA systems has a specific climate-related job whereby it must maintain or adjust an environmental parameter, based on external factors, to ensure production requirements are met (van Iersel 2017). While variables such as production materials (e.g., substrate) and nutrients can be heavily controlled or have little influence in

microgreen production, factors such as light and temperature are not as easily manipulated. This difficulty in control is partially due to confounding, external aspects such as weather (van Iersel 2017; Poncet et al. 2012). Because of this, CEA operations producing the same microgreen crops may have to approach environmental control differently to achieve success in their region.

Information on external influence of environmental factors is often collected over time on a single Programmable Logic Controller (PLC) (Li et al. 2017). Such technology allows growers and scientists alike to execute changes to their environment based on real-time data, or even set their CEA operation to automatically adapt (Park et al. 2022). As mentioned previously, two crucial environmental factors for the production of any plant in a controlled environment are light and temperature. While sensing technologies can help control such parameters, further exploration into both light and temperature from a species-specific standpoint is essential to best optimize production. Microgreens are sensitive to light and temperature parameters due to their young stage of growth. Nevertheless, there is a lack of scientific exploration into the interaction between light and temperature during microgreen production. Given light and temperature can be controlled separately in CEA, there is an opportunity to explore how combinations found in nature and anthropogenic formulas could benefit the CEA industry.

Light and temperature are especially important when considering the future of microgreen production. The most common practice for scaling up microgreen production is to vertically replicate homogeneous growing sections, with repetitive equipment stacked (Goodman and Minner 2019). Because of this vertical design, microclimates are inevitable, including both temperature and light distribution.

Microgreens are commonly grown using hydroponic systems in controlled environments. Seeds are sown on a thin layer of substrate composed of either soil, coir, or natural or synthetic

fibrous material to allow for the establishment of initial rooting for (Moraru et al. 2022; Puguh Bintang Pamungkas et al. 2023). Typically, sowing densities in systems or flats are determined based on the weight of seeds, providing consistency in yield (Kyriacou et al. 2016). While microgreens are commonly grown with no fertilizer inputs, a low rate of nitrogen has been found to be beneficial throughout production (Moraru et al. 2022). Adding fertilizer to any plant production will alter the acidity of the substrate used as well as the concentration of nutrients, which can be quantified by pH and electrical conductivity (EC), respectively. While slight changes in pH and EC are common throughout production, targets are generally set and maintained. A common pH and EC target for microgreen production is 5.5-6.5 and 0.75 $mS \cdot cm^{-1}$, respectively, although specifics are species-dependent (Kyriacou et al. 2016).

1.4 Lighting Optimization for Microgreens

While supplemental lighting is often necessary in greenhouse settings to achieve a uniform light environment year-round, microgreens are commonly grown under sole-source lighting in indoor environments. Thus, the production of microgreens in indoor environments necessitates the use of electric lighting. Industry standard lighting sources, such as high-pressure sodium (HPS) lamps, have received recent competition from light-emitting diodes (LEDs) as a viable, energy efficient lighting alternative. Light-emitting diodes have become commonplace in the CEA industry due to their many benefits compared to traditional lighting systems. For example, LEDs produce very little latent heat, allowing greater control of ambient temperature in the production environment and facilitating the use of multi-layered systems where electric fixtures are placed in close proximity to the crop canopy (van Iersel 2017). Additional benefits of LEDs include spectral control and improved electrical efficacy (Morrow 2008).

Similar to labor, electric lighting has a high price point, with a demand up to 40% to 50% of annual operational costs (Zeidler et al. 2017). Therefore, practices to reduce the overall energy input for horticultural applications using electric lighting pose a promising area of research, as decreasing costs related to one of the more expensive aspects of CEA is crucial for further industry expansion (Park et al. 2022). Expanding lighting research also creates a platform for plant-specific light spectra to be explored, defined, and used in industry (Choe et al. 2018).

1.4.1 Photosynthetically Active Radiation (PAR)

Photosynthetically active radiation (PAR; 400-700 nm) is the range of wavelengths within the electromagnetic spectrum that plants use for photosynthesis (Zhen et al. 2022). Photosynthetically active radiation encapsulates intensities of light that are best defined by peak wavelengths or ranges represented to humans as the colors blue (400-500 nm), green (500-600 nm), and red (600-700 nm) (Alados et al. 1996; Zhen et al. 2021). While the sun produces a broader spectrum of wavelengths, producing PAR-specific photons via electric lighting ensures efficient use of electricity by only producing wavelengths found necessary for driving photosynthetic activity (Zhen et al. 2021).

For horticultural applications, we measure instantaneous light intensity within the PAR range as photosynthetic photon flux density (PPFD; $\mu mol \cdot m^{-2} \cdot s^{-1}$) (Zhen et al. 2021; Pérez-Bermúdez and Rognoni Martínez 2023). Photosynthetic photon flux density integrated over a 24-hour period is referred to as the daily light integral (DLI; $mol \cdot m^{-2} \cdot d^{-1}$) (van Iersel 2017). Thus, DLI is a function of PPFD and photoperiod. For greenhouse production, DLI varies due to a variety of factors including the time of year, weather, geographic location, and cardinal orientation (van Iersel 2017). For such reasons, supplemental lighting is an essential solution to less than desired DLIs in greenhouse settings. While electric lighting can be used in combination

with sunlight to achieve a target DLI in the greenhouse, for indoor production using sole-source lighting (light only provided from electric lamps), a PPFD can be targeted to maintain a precise DLI. It is understood through research by Gerovac et al. (2016) that an increase in DLI can lead to an increase biomass for multiple *Brassica* microgreen species. For example, when comparing microgreens grown under a DLI of 6 or 18 $mol \cdot m^{-2} \cdot d^{-1}$, fresh weight increased with increased DLI for the microgreen species mustard (*Brassica juncea* 'Garnet Giant') and mizuna (*Brassica rapa* L. var. *japonica*) by 34%, and 15%, respectively (Gerovac et al. 2016).

Brassica microgreens have shown to increase in phytochemical concentration when exposed to increasing PPFDs ranging from 110 $\mu mol \cdot m^{-2} \cdot s^{-1}$ to 545 $\mu mol \cdot m^{-2} \cdot s^{-1}$ (Samuolienė et al. 2013). While higher PPFDs resulted in increased phytochemical concentration, hypocotyl elongation and leaf area expansion were limited, leading to the conclusion that lower PPFDs (330 to 440 $\mu mol \cdot m^{-2} \cdot s^{-1}$) for *Brassica* microgreens are optimal (Samuolienė et al. 2013). While Samuolienė et al. (2013) established that a lower PPFD was optimal for *Brassica* microgreens, the LED spectra used in that experiment consisted of two red peak wavelengths (638 and 665 nm) and one blue peak wavelength (445 nm); this focus on red:blue sole-source lighting lacks many naturally-occurring wavelengths that may benefit photochemistry and provide a wholistic understanding of optimal PPFD for microgreens production. Fortunately, work by Gerovac et al. (2016) continued avenues of the previously mentioned research and concluded that both light intensity and quality, with the inclusion of far-red (FR) wavelengths, are essential factors in microgreen production, and sometimes even interact depending on species. At a PPFD of 105 $\mu mol \cdot m^{-2} \cdot s^{-1}$, Gerovac et al. (2016) compared spectrums including blue:red, blue:green:red, and blue:red:far-red and found that the inclusion of green and FR light resulted in increased hypocotyl length for kohlrabi (*Brassica oleracea* L. var. *gongylodes*) microgreens; however,

responses were species-specific and dependent on PPFD (Gerovac et al. 2016). Such work exposes the necessity for research to develop species-specific lighting protocols for growers (Gerovac et al. 2016).

1.4.2 Far-red Radiation

Far-red (FR) wavelengths (700-800 nm) are longer and lower energy than others traditionally included within the definition of PAR (Zhen et al. 2022). However, recent studies have validated the need for FR wavelengths to be considered photosynthetically active, forming an interim term, extended PAR (ePAR) (Zhen et al. 2021). For example, Zhen and van Iersel (2017) explain that FR light can increase the quantum yield of photosystem II (PSII) and leaf photosynthetic rate when used in combination with red (600-700 nm) wavelengths (Park and Runkle 2018; Zhen and van Iersel 2017). While FR light is naturally occurring, its presence in modern LED supplemental lights is often lacking or poorly understood and in need of further research (Zhen et al. 2021). As a reference, the red to FR light ratio (R:FR) is commonly used to describe the presence of FR light in a natural or controlled environment, meaning the more FR light included in the spectrum, the lower the R:FR.

It is understood that FR light must be provided in combination with red wavelengths to have a photosynthetic effect (Zhen and van Iersel 2017). It was found by Zhen and van Iersel (2017) that lettuce grown under a low R:FR (via supplemental FR light) caused an improved efficiency of electron transport for PSII and decreased the quantity of light lost as heat (reduction in non-photochemical quenching) (Zhen and van Iersel 2017). This is due to FR light over-exciting Photosystem I (PSI), which allows PSII to operate at a higher rate. Since FR light directly excites PSI, and photosynthesis can only operate as efficiently as the lagging

photosystem, the inclusion of FR increases photochemical efficiency by ensuring both PSI and PSII are equally excited (Zhen and van Iersel 2017).

Far-red light is abundant in inter-canopy settings as other wavelengths are more readily absorbed by leaves above (Smith and Whitelam 1997). This, as well as shorter, higher-energy wavelengths losing energy via absorption, transmission, and reflection from the above canopy leaves behind an abundance of lower energy wavelengths such as FR light (Smith and Whitelam 1997). As a result, a low R:FR is common in shaded environments, resulting in naturally occurring phenomena known as shade avoidance responses (SAR) (Wang et al. 2015). Shade avoidance responses occur when plants are competing for light in either a low PPFD or low R:FR environment, such as inter-canopy settings or cloudy days (Kusuma and Bugbee 2023; Park and Runkle 2018; Smith and Whitelam1997). Shade Avoidance Response can also result from a high PPFD so long as R:FR is low (Park and Runkle 2018). While characteristics of SARs are species-dependent, common responses include increased stem and petiole elongation, leaf hyponasty, increased or inhibited leaf expansion, and reduced pigmentation (Wang et al. 2015). The contrasting responses, such as increased or inhibited leaf expansion, and species-specificity make SAR characterization for controlled environment production difficult.

The variation in the photomorphogenesis that an abundance of FR light (low R:FR) brings is often discussed in negative rhetoric. This is due to the way in which SARs influence plants grown to full size. A notable recorded SAR is stem elongation as it is easy to identify and occurs rapidly (Smith and Whitelam 1997). Still, SAR could be beneficial for its provocation of stem elongation for practices such as grafting (Chia and Kubota 2010) or promotion of leaf area expansion to improve photon capture and light use efficiency (Park and Runkle 2018). Further, such plasticity could be used to increase the overall area and height of a plant faster than

traditionally expected (Casal 2012). Because of this, SAR may be advantageous in crowded environments (Casal 2012), such as densely sown microgreens. For certain production methods, such as those utilized for microgreens, providing strategically implemented FR light to induce SAR may increase growth rate and quality. Far-red wavelengths are already understood to promote seedling growth and flowering in low light environments for petunia *(Petunia* × *hybrida*) (Park and Runkle 2018). Mah et al. (2018) found an increased stem length in marigolds (*Tagetes erecta*) and petunias (*Petunia* × *hybrida*) under low R:FR of 0.7 compared to higher R:FR of 1.1. Nevertheless, such a range is very narrow, indicating how a better understanding of FR could lead to a higher level of control in horticultural production (Mah et al. 2018). Such phenotypic responses are attributed to how plants perceive their surrounding light environment.

Plants have an abundance of photoreceptors that respond to different qualities and intensities of light (Wang and Folta 2013). For instance, there are photoreceptors that specifically absorb red and FR wavelengths, resulting in a variety of plant responses including photomorphology and flowering (Wang and Folta 2013). Specifically, phytochrome b (Phy_B) is the photoreceptor responsible for sensing differences in the R:FR (Ballaré and Pierik 2017). Phy_B has two interconvertible states that it resides in, depending on the R:FR; an active FR absorbing form and an inactive red absorbing form (Ballaré and Pierik 2017). Phy_B is converted into the biologically active form (P_{fr}) in the presence of red light, and the biologically inactive form (P_R) in the presence of FR light or darkness (Ballaré and Pierik 2017). When a plant is exposed to a low R:FR, the biosynthesis of growth-related hormones is stimulated, resulting in characteristics of the SAR (Ballaré and Pierik 2017). Through a holistic approach to shade signaling, Ballaré and Pierik (2017) found that plants in the Phy_B active (P_{fr}) state will remain there unless exposed to an abundance of FR light or darkness. Further, plants in the active Phy_B state have the

inhibition of stem elongation, contrary to the inactive form of Phy_B (Hendricks and Borthwick 1967). As different plant species have different light requirements and different tolerances to juxtaposing plants, there is variation in how SAR is expressed. It is important to note that Phy_B is also thermoregulatory, indicating that such reactions brought on by the phytochrome may also be influenced by temperature (Thingnaes et al. 2008). Characteristics of SAR open an area of exploration into how light specifications can be utilized for high quality plant production.

To understand how FR light can best be used in industrial practices, the R:FR ratio must be dissected and understood from a quality and quantity perspective. Recently there have been experiments validating and evaluating various ratios of FR in supplemental spectra. The R:FR of 0.4 has been used in multiple experiments (Lund et al. 2007; Heraut-Bron et al. 1999) consistently resulting in stretched characteristics such as stem elongation and leaf area increase compared to a higher R:FR across many species. For example, Heraut-Bron et al. (1999) found that leaf area of white clover (*Trifolium repens*) increased under a low R:FR of 0.4 compared to 2.4, but this SAR was dependent on PPFD as this increase was observed under a PPFD of 320 $\mu mol \cdot m^{-2} \cdot s^{-1}$ but not 110 $\mu mol \cdot m^{-2} \cdot s^{-1}$. Far-red light is understood to induce SAR even when provided for a short duration of 30 minutes as end-of-day (EOD) lighting (Jeong et al. 2024; Lund et al. 2007). For example, poinsettia (*Euphorbia pulcherrima* 'Christmas Spirit') grown using EOD red light were 54% shorter in height compared to those grown with EOD FR light (Islam et al. 2014). Lund et al. (2007) compared a 30-minute EOD light treatment with a R:FR of 0.4 to a control R:FR of 2.4 and found that plant height increased for chrysanthemum (*Chrysanthemum morifolium* var. 'Coral Charm') (Lund et al. 2007).

Some research into FR light has shown direct application to industry practices, such as increasing the quality of tomato rootstock via a low R:FR ratio (Chia and Kubota 2010).

Specifically, Chia and Kubota (2010) found that utilizing EOD lighting with a low R:FR resulted in elongated hypocotyls of tomato rootstock cultivars Aloha (*Solanum lycopersicum*) and Maxifort (*Solanum lycopersicum* × *Solanum habrochaites*), when compared to control groups not receiving supplemental EOD FR-right lighting. As discussed previously, Zhen and Bugbee (2020) have argued for the inclusion of FR light in the new definition of ePAR, pushing for a motive to redefine the parameters of PAR. An alteration in the definition of PAR would bring a major change in light parameters for CEA, further justifying a need to fully understand species-specific responses to FR light both to capitalize on beneficial and prevent negative SAR responses.

1.5 Temperature Influence on Development of Microgreens

Temperature plays an essential role in the growth rate of plants and is often characterized in CEA by using day (DT) and night temperatures (NT) to calculate DIF. DIF is the acronym used to characterize the difference in DT/NT, resulting in either a positive, negative, or zero value (Ballaré and Pierik 2017). A positive DIF (+DIF) is associated with promoting growth in ways such as increased elongation. On the contrary, a negative DIF (-DIF), higher NT and lower DT, suppresses elongation (Ballaré and Pierik 2017; Kong et al. 2023). DIF also helps growers understand their energy usage, as target temperatures vary throughout both the day and time of year (Park et al. 2022).

Microgreens generally grow optimally within an air temperature range of 20 to 25 ºC (Gerovac et al. 2016; Moraru et al. 2022). While there is a tolerance to what temperature microgreens can be grown at, it is understood that growth rate increases with warmer temperatures (Kong et al. 2023). For example, Kong et al. (2023) found that mustard

microgreens have a higher rate of growth at warmer temperatures, independent of varying light treatments.

It is understood that Phy_B plays a role in the thermoperiodic response of plants (Thingnaes et al. 2008). Given Phy_B's preexisting role in photomorphogenesis, a compelling argument can be made for experiments that explore the relationship between FR light and temperature responses. Recently, Jeong et al. (2024) concluded that FR light and temperature interactively regulate growth and morphology of lettuce (*Lactuca sativa*) 'Rex' and basil (*Ocimum basilicum*) 'Genovese' grown at a constant PPFD of 250 $\mu mol \cdot m^{-2} \cdot s^{-1}$. Specifically, these authors tested varying percentages of FR replacement (0%, 10%, and 20%) at either a constant temperature (20, 24, and 28 ºC), +DIF (28/20 ºC), or -DIF (20/28 ºC) and found that leaf expansion from the inclusion of FR light was promoted at 20 and 24 ºC but inhibited at 28 ºC (Jeong et al. 2024). Temperature is also known to regulate phytochrome-mediated responses such as reduced stem elongation at colder air temperatures (Jeong et al. 2024). Finding an optimal temperature and light regime that work synergistically could reduce production time for growers, resulting in larger profit margins.

1.6 Summary

In summary, there is a pressing need for research exploring the interactions between FR light and temperature. To do so, species-specific SARs need to be documented under varying light and temperature environments. Induced SAR to benefit microgreen production may reduce the cost of production by decreasing production time (Lanou et al. 2022) but could also come at the cost of reduced pigment concentration discussed by Wang et al. (2015). By evaluating the role of FR light in SAR, alongside possible interactions with ambient temperature, strategies may be developed whereby temperature-induced stress could compensate for potential reduction in

pigmentation from FR light (Kong et al. 2023). We first investigate the use of FR light supplementation and replacement at two ambient temperatures to explore a possible interaction between light and temperature to enhance *Brassica* microgreens production, and then investigate a possible "dynamic" lighting and temperature strategy to enhance both morphological characteristics and nutritional quality of red cabbage microgreens.

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CHAPTER 2. FAR-RED LIGHT SUPPLEMENTATION AND REPLACEMENT RESULTS IN SHADE AVOIDANCE RESPONSES AND INCREASED BIOMASS FOR THREE BRASSICA MICROGREEN SPECIES INDEPENDENT OF AMBIENT AIR TEMPERATURE

2.1 Introduction

Microgreens production is a fast-growing market due to their nutritional benefits and ease of production, inspiring a wave of horticultural entrepreneurs filling restaurants and grocery stores alike (Verlinden 2019). Microgreens are generally harvested 7 to 14 days after sowing, with a final plant product consisting of a hypocotyl, cotyledons, and one to two true leaves (Choe et al. 2018; Verlinden 2019). Common plant species utilized for microgreens production include arugula (*Eruca sativa)*, kohlrabi (*Brassica oleracea*), mizuna (*Brassica rapa* var. *nipposinica*), mustard (*Brassica juncea),* radish (*Raphanus sativus*), red cabbage *(Brassica oleracea* var. *capitata*) (Choe et al. 2018). Required production inputs for microgreens in controlled environments are typically limited (Kyriacou et al. 2016), with recommendations often including a low photosynthetic photon flux density (PPFD; $\mu mol \cdot m^{-2} \cdot s^{-1}$) (Kong et al. 2023) and minimal or no fertilizer (Moraru et al. 2022). In terms of lighting, light-emitting diodes (LEDs) have played a significant role in facilitating the implementation of indoor microgreens production due to their low emission of radiant heat, generally high electrical efficacy, and potential to manipulate both spectrum and PPFD to achieve desired crop requirements (Morrow 2008).

As mentioned, previous research has shown that many species of microgreens can be produced using a minimal PPFD throughout production in a controlled environment. It is documented that fresh and dry weight of various microgreen species increase as PPFD increases (Jones-Baumgardt et al. 2019). Jones-Baumgardt et al. (2019) found that under a light spectrum consisting of only red (600-700 nm) and blue (400-500 nm) wavelengths, the fresh weight of

Brassica microgreens increased as PPFD increased from 100 to 600 $\mu mol \cdot m^{-2} \cdot s^{-1}$; however, the efficiency by which light led to further increases in biomass heavily dropped off in effectiveness after 300 $\mu mol \cdot m^{-2} \cdot s^{-1}$, indicating that a lower PPFD is ideal for efficient microgreen production (Jones-Baumgardt et al. 2019). It is also recorded that the nutritional content of microgreens is impacted by PPFD throughout production. Discussed by Lanoue et al. (2022), using a constant daily light integral (DLI; $mol \cdot m^{-2} \cdot d^{-1}$) of either 14 or 21 $mol \cdot m^{-2} \cdot d^{-1}$ (each DLI tested at both photoperiods) it was concluded that phenolic concentrations were either maintained or increased under a low constant (24 hours) PPFD compared to microgreens receiving 16 hours of light per day. Further, Liu et al. (2022) discovered that soluble protein content in cabbage (*Brassica oleracea*) microgreens was highest under a PPFD of 90 $\mu mol \cdot m^{-2} \cdot s^{-1}$ among PPFD values ranging from 30 to 90 $\mu mol \cdot m^{-2} \cdot s^{-1}$. This study was conducted on multiple species, with kale (*Brassica alboglabra*) microgreens displaying the highest soluble protein content under a PPFD of 70 $\mu mol \cdot m^{-2} \cdot s^{-1}$, providing insight to species-specific responses (Liu et al. 2022).

In addition to PPFD, the quality of LED lighting has also been shown to manipulate microgreen growth and morphology. Far-red (FR) light (700-800 nm) is of interest due to its role in flowering, photomorphology, and photochemistry. Specifically, FR wavelengths ranging from 700-750 nm are of interest, as they are understood to have a photosynthetic effect in combination with current wavelengths defined as photosynthetically active radiation (PAR; 400-700 nm) (Kusuma and Bugbee 2023; Zhen et al. 2021). Thus, Zhen et al. (2022) argue for the inclusion of FR wavelengths into the definition of PAR, resulting in the term 'extended photosynthetically active radiation' (ePAR; 400-750 nm).

Additionally, the ratio of red to FR light (R:FR) impacts photomorphology, particularly with a low R:FR a variety of shade avoidance responses (SAR) occur (Hooks et al. 2022; Mah et

al. 2018; Smith and Whitelam 1997). For context, FR light is abundant in shaded areas, resulting in a low R:FR which signals a plant to adapt morphology in attempt to grow towards a perceived and increased abundance of light to increase photon capture. Characteristic SAR in plants includes hypocotyl elongation, cotyledon and true leaf extension, a reduction in leaf mass area, and a potential reduction in pigmentation per unit leaf area (e.g., chlorophyll, anthocyanin) (Gerovac et al. 2016; Kong et al. 2023). For example, Mah et al. (2018) explored R:FR of either 0.7 or 1.1 on the growth and morphology of bedding plants and concluded that the low R:FR of 0.7 increased plant height by 11%, 22%, and 32% for the species marigold (*Tagetes erecta* 'Antigua Orange'), petunia (*Petunia* x *hybrida* 'Duvet Red'), and calibrachoa (*Calibrachoa* x *hybrida* 'Kabloom Deep Blue'), respectively, compared to the R:FR of 1.1. Studies such as this provide insight not only regarding the impact of R:FR on plant morphology, but also the influence of species on SAR (Liu et al. 2022; Mah et al. 2018). While previous research generally focuses on the negative impacts of SAR on crop production, SAR characteristics may be deemed beneficial for specialty crops such as microgreens. For example, an increase in leaf area resulting from a low R:FR could lead to increased photon capture, resulting in increased light use efficiency for electrical fixtures and reduced production time (Park and Runkle 2018). For microgreens in particular, which consist mostly of a hypocotyl, hypocotyl elongation under a low R:FR could potentially decrease production time and increase biomass accumulation.

The photoreceptor responsible for perceiving the quantity and qualities of red and FR light is phytochrome b (Phy_B) (Smith and Whitelam 1997). Phy_B exists in two interconvertible forms; the active, FR absorbing state (P_{fr}) and the inactive, red absorbing state (P_r) (Ballaré and Pierik 2017). Thus, under a high R:FR, P_{fr} is in abundance inhibiting SAR; whereas under a low R:FR, SAR are common due to a shift to the inactive red absorbing state (P_r) (Ballaré and Pierik

2017; Zhen et al. 2022). There is also a thermoregulatory aspect to Phy_B, as it is always converting back to P_r at a rate dependent on air temperature (Ballaré and Pierik 2017; Thingnaes et al. 2008). Such characteristics of Phy_B indicate that morphological responses under sole-source LED lighting may also be dependent on production air temperature. Recently Jeong et al. (2024) determined that temperature and FR light regulate plant morphology interactively, by assessing lettuce 'Rex' (*Lactuca sativa*) and basil 'Genovese' (*Ocimum basilicum*) grown under increasing intensities of FR light at an ambient temperature ranging from 20 ºC to 28 ºC. It was concluded that FR light was most effective at increasing leaf area when it was provided at a high percentage of the total spectrum (up to 20%) under an air temperature of 20 or 24 ºC, whereas at an air temperature of 28 ºC, FR light had little impact on leaf area (Jeong et al. 2024). Interactions between temperature and light on photomorphogenesis are not limited to FR wavelengths, as discussed by Kong et al. (2023), who analyzed arugula microgreens grown at an ambient air temperature of 28 ºC under monochromatic blue (450 nm peak) or red (670 nm peak) LED sole-source lighting. It was concluded that hypocotyl elongation increased by 37% under blue compared to red light at a PPFD of 110 $\mu mol \cdot m^{-2} \cdot s^{-1}$ (Kong et al. 2023). Further, the degree of elongation responses appeared to be driven by ambient air temperature, with hypocotyl elongation increasing by 160% under blue compared to red light at a PPFD of 110 $\mu mol \cdot m^{-2} \cdot s^{-1}$ when the air temperature was decreased to 18 ºC (Kong et al. 2023). This indicates a need for further exploration into temperature-driven light responses and improved understanding of wavelengths bordering what we consider to be the traditional range of PAR.

To date, there is limited information on how light spectra and temperature interactively affect plant growth and morphology. Therefore, the objective of this study was to investigate the benefit of reducing air temperature as well as supplementing with FR light (700-750 nm) to

enhance the morphology and phytochemical concentrations of three *Brassica* microgreen species. We hypothesized that the inclusion of FR light in the spectrum, either by supplementation or replacement, would lead to documented SARs (e.g., increased hypocotyl elongation and leaf area) as well as increase biomass accumulation, and that the magnitude of these responses may be dependent on production air temperature. Additionally, we hypothesized that a reduction in air temperature would lead to an increase in microgreen phytochemical concentrations (e.g., anthocyanin). While provoked hypocotyl elongation in FR rich conditions may not be beneficial for fully developed plants, such SARs may decrease production time in a crop characterized by a stage of growth primarily consisting of a hypocotyl. If so, growers could benefit from a reduced production time resulting in increased profit margins.

2.2 Materials and Methods

2.2.1 Plant Material, Culture, and Germination

Seeds of mustard (*Brassica juncea* 'Garnet Giant'), kohlrabi (B*rassica oleracea* var. *gongylodes*), and red cabbage (*Brassica oleracea* var. *capitata*) were sown on 16.5 x 25.4 cm (419.4 cm^2) sections of rockwool, a fibrous soilless substrate, (Grodan Rockwool Micro-Greens Cress Propagation Mat; Milton, Ontario, CA). Seed densities of 2.67, 3.17, and 3.50 g per rockwool section were sown on the substrate mats for mustard, kohlrabi, and red cabbage, respectively. The substrate was presoaked with tap water prior to sowing, and sections were immediately placed in walk-in growth chambers after seeding (GR64; Conviron, Winnipeg, Canada).

After sowing, mat sections were placed in trays (52 × 26 × 6 cm) without drainage holes, along with one cup of water-soluble fertilizer (pH 5.5-6.5; EC 0.75 $mS \cdot cm^{-1}$) using 13N–0.9P–10.8K Plug Special (Plant Marvel; Chicago Heights, IL) providing (in $mg \cdot L^{-1}$) 150 nitrogen (N),

23 phosphorus (P), 150 potassium (K), 69 calcium (Ca), 34 magnesium (Mg), 0.15 boron (B), 0.07 copper (Cu), 0.75 iron (Fe), 0.37 manganese (Mn), 0.07 molybdenum (Mo), and 0.37 zinc (Zn). Trays were then covered with a humidity dome until germination reached at least 50%, which occurred on day three for mustard and kohlrabi, and day four for red cabbage. For mustard and kohlrabi germination, temperature and humidity targets were set to 21 ºC and 55%/65% (D/N) respectively, under a homogenous PPFD of 165 $\mu mol \cdot m^{-2} \cdot s^{-1}$ with a 16-h photoperiod (600-2200 HR). Due to the shared nature of the two species protocols, mustard and kohlrabi trials were conducted concurrently. Conversely, red cabbage seed germinated in darkness at temperature and relative humidity setpoints of 24 ºC and 55%/65%, respectively.

2.2.2 Growth Chamber Environment

Three (mustard and kohlrabi) or four (red cabbage) days after sowing, humidity domes were removed, and mat sections transferred to webbed trays (52 × 26 × 6 cm). Webbed trays were then placed in ebb and flow tables and irrigated every 3 hours using the same fertilizer described above with a target EC of 0.75 $mS \cdot cm^{-1}$. A target pH of 5.5 to 6.0 was maintained by adding phosphoric acid (pH down; General Hydroponics, Santa Rosa, CA). pH and EC were measured using a handheld probe (Growline H19814; Hanna Instruments, Woonsocket, RI) and system adjustments were made as needed every two days.

Growth chambers consisted of four counterbalanced light canopies (RAY44 Physiospec Indoor and FR fixtures; Fluence, Austin, TX), of which three were used per trial. Each light canopy resulted in an individual light treatment, which varied in both PPFD and spectrum. Light contamination between treatments was minimized through curtains which divided each chamber into two sections. Lighting treatments from the three light canopies included the following: PPFD of 165 $\mu mol \cdot m^{-2} \cdot s^{-1}$ (PAR_{165}); PPFD of 200 $\mu mol \cdot m^{-2} \cdot s^{-1}$ (PAR_{200}); and PPFD of 165

$\mu mol \cdot m^{-2} \cdot s^{-1}$ + 35 $\mu mol \cdot m^{-2} \cdot s^{-1}$ of FR light (PAR_{165}+FR_{35}). Thus, comparisons between PAR_{165} and PAR_{165}+FR_{35} allow for an evaluation of supplemental FR light while comparisons between PAR_{200} and PAR_{165}+FR_{35} provide a means to evaluate the impacts of ePAR. With each growth chamber separated by a curtain, PAR_{165}+FR_{35} was isolated from PAR_{165} and PAR_{200} to ensure FR light contamination between treatments was minimized. To achieve homogeneity with sole-source lighting, light was measured at its point of incidence to create a homogenous light plane. This was done by dimming the LED fixtures to a set output percentage, as well as the physical distance of the lights from the plant. Light treatments were established at the beginning of each trial using a spectrometer (LI-180; Li-COR Biosciences, Lincoln, NE), with mean ± SD of various light parameters characterizing each treatment included in Table 1. To further ensure uniform light distribution under each light canopy, trays were rotated every other day.

Two walk-in growth chambers (GR64; Conviron) were used for this study, with the light treatments described above established within each. Each growth chamber maintained an air temperature setpoint of either 18 or 21 ºC for each trial post-germination, with a temperature mean ± SD of 19.1 ± 1.1 ºC and 21.5 ± 0.6 ºC, respectively (HOBO UX100-011A, Onset Data Loggers, Bourne, MA). Relative humidity mean ± SD for the air temperature setpoints of 18 and 21 ºC were 61.4% ± 7.1% and 57.9% ± 8.1%, respectively (HOBO UX100-011A, Onset Data Loggers, Bourne, MA). A total of six treatment combinations were evaluated for this experiment, with three mats for each species evaluated under each treatment. The experiment was repeated with the temperature setpoints swapped between the two growth chambers, resulting in a total of six replications (mats) per species for each treatment combination.

2.2.3 Microgreen Data Collection

Three forms of data collection were conducted for this experiment: non-destructive through-trial height, destructive data on individual microgreens, and destructive data on mats. Non-destructive through-trial height (cm) was taken every two days, starting on day five and ending on day 11, for four total measurements taken throughout each trial. One measurement from each side of a mat was recorded per measurement period, resulting in four total measurements per mat per day. Given there were three replications (mats) per light bay per trial, the most homogenous mat was picked on day 5 of every trial and used the duration of the measurements. Measurements were taken by hand, using a handheld ruler (Cat. No. 09-016; Fisher Scientific, Waltham, MA). The four measurements per mat per day were averaged for a single measurement per mat.

Individual microgreen destructive data was collected 12 d after germination for all species, with day sowed considered as day 0, and day 12 as the final day of growing. Five microgreens were selected at random from each mat section and data was collected on fresh weight (g; Analytical Balance ME54E; Mettler Toledo Ltd, Columbus, OH), hypocotyl length (cm), hypocotyl diameter (mm; *Fisherbrand* Traceable; Thermo Fisher Scientific Waltham, MA) width (mm), leaf number, and leaf area (cm^2; Li-COR LI3100C Area Meter; Li-COR Biosciences, Lincoln, NE). Microgreens were then dried at 70 °C for at least 5 days to determine the dry weight (mg) of each using an analytical microbalance (Analytical Balance ME54E; Mettler Toledo Ltd). Fresh and dry weight data were taken on the remaining microgreens for each mat, using the same drying methodology as individual microgreen dry weight.

2.2.4 Statistical Analysis

Experimental trials were conducted between May 20, 2023, and September 30, 2023, at the CSU Spur Campus in Denver, Colorado. This experiment was a randomized complete block design with light-temperature treatments (6 levels) as treatment factors and repetition (2 levels) as a blocking variable. The blocking variable (repetition) was included in the analysis, with the temperature conditions within each chamber switched between repetitions. Destructive data analysis was derived from six replications (both repetitions), whereas the data reported for full mat analysis and through-trial measurements came only from three replications (the first repetition). Microgreen species were independently analyzed to mitigate species-specific influence on the understanding of phenomena.

We evaluated the relationship between light and temperature through a two-way ANOVA, using R statistical software (Lenth, 2021; R Core Team, 2021). Light and temperature were kept together in the statistical model for every mode of analysis but analyzed as their own main effects separately. Light and temperature were separated for estimated marginal means, and confidence letter display, created from pairwise comparison constructed using Tukey's honestly significant difference (HSD) test at $P \leq 0.05$.

2.3 Results

Limited interaction between light and temperature was observed across experimental trials. Therefore, light and temperature were analyzed and reported as separate main effects.

2.3.1 Individual Microgreen and Full Mat Destructive Data - Main Effect: Light

Hypocotyl length, hypocotyl diameter, leaf number, leaf area, fresh weight, and dry weight were generally similar for all microgreen species under PAR_{200} compared to PAR_{165}

(Table 2). The only exceptions were fresh and dry weight for kohlrabi, whereby PAR_{200} resulted in a 60% and 34% increase compared to PAR_{165} (Table 2). However, the inclusion of 35 $\mu mol \cdot m^{-2} \cdot s^{-1}$ of FR light (PAR_{165}+FR_{35}) generally resulted in increased hypocotyl length, hypocotyl diameter, leaf area, fresh weight, and dry weight for all species compared to both PAR_{165} and PAR_{200} (Table 2). For example, hypocotyl length of red cabbage under PAR_{165}+FR_{35} was 94% greater than both PAR_{165} and PAR_{200} (Table 2). Similarly, fresh weight was 60%, 51%, and 38% greater under PAR_{165}+FR_{35} compared to PAR_{165} for mustard, kohlrabi, and red cabbage, respectively (Table 2). Similar biomass data between PAR_{165}+FR_{35} and PAR_{200} was observed for kohlrabi and red cabbage fresh and dry weights; fresh and dry weights were otherwise greatest under PAR_{165}+FR_{35} for mustard (Table 2).

For mustard microgreens, full mat fresh and dry weights were similar for all light treatments (Table 3). Increased fresh and dry weights were observed for kohlrabi microgreen mats under PAR_{165}+FR_{35} and PAR_{200} compared to PAR_{165}, and the highest full mat fresh and dry weights were observed under PAR_{165}+FR_{35} for red cabbage (Table 3). For example, red cabbage full mat fresh weight under PAR_{165}+FR_{35} was 46% and 27% greater compared to PAR_{165} and PAR_{200}, respectively (Table 3).

2.3.2 Individual and Full Mat Destructive Data - Main Effect: Temperature

Production at 21 ºC led to increased hypocotyl length, hypocotyl diameter, leaf number, leaf area, fresh weight, and dry weight compared to 18 ºC for all three microgreen species. For example, hypocotyl length for mustard, kohlrabi, and red cabbage was 36%, 38%, and 19% greater, respectively, under 21 ºC compared to 18 ºC (Table 4). Responses of the full microgreen mats followed a similar trend, whereby the greatest fresh and dry weights were observed under 21 ºC compared to 18 ºC for all three species (Table 5). For example, mustard, kohlrabi, and red

cabbage displayed 107%, 125%, and 191% greater fresh weights under 21 ºC compared to 18 ºC, respectively (Table 5).

2.3.3 Through-trial Measurements

Regardless of temperature and microgreen species, a trend of increased hypocotyl elongation (height) was observed under PAR_{165}+FR_{35} compared to PAR_{165} and PAR_{200} as early as day 7 (Fig. 1). For example, at both 18 and 21 ºC, mustard and kohlrabi grown under PAR_{165}+FR_{35} showed increased hypocotyl elongation compared to both PAR_{200} and PAR_{165} by day 7, while differences between PAR_{200} and PAR_{165} were not observed (Fig. 1A-D). For red cabbage at 18 ºC, hypocotyl length was greater under PAR_{200} than PAR_{165} by day 7, and further, hypocotyl length under PAR_{165}+FR_{35} was greater than PAR_{200} by day 7 at both 18 and 21 ºC (Fig. 1E-F).

2.4 Discussion

Shade avoidance response characteristics were present across all three microgreen species under PAR_{165}+FR_{35}. Specifically, all species saw an increase in hypocotyl length and leaf area with the addition of FR light (Table 2), which is a well-documented SAR (Hooks et al. 2022; Kong et al. 2023; Samuolienė et al. 2013 et al.). While shade avoidance is an established phenomenon, the combinations of species-specific expressions and R:FR that cause them are left ambiguous (Liu et al. 2022). Because of this, SAR can often contradict one another when assessing various species at the same R:FR, resulting in a collection of different results for studies evaluating FR light. For instance, Hooks et al. (2022) concluded that hypocotyl elongation is accompanied by a reduced cotyledon area in *Brassica* microgreens, whereas Mah et al. (2018) concluded the same finding only for certain bedding plant species, specifically

marigold and petunia. Thus, our findings also differ from previous research as both an increase in hypocotyl length and leaf area (cotyledon plus true leaves) was observed across all three species under PAR_{165}+FR_{35} (Table 2). One possible explanation for this discrepancy is regarding the R:FR used in the current study, as PAR_{165}+FR_{35} resulted in the relatively high R:FR of 2.0 with FR wavelengths encompassing ~17% of the provided spectrum (Table 1). As a comparison, the R:FR of natural sunlight is considerably lower at ~1.3 (Zhen et al. 2022). Thus, PAR_{165}+FR_{35} did not necessarily have a low R:FR like the two previously mentioned studies, rather an abundance of FR light added to an adequate PPFD for microgreen production. This understanding helps to explain other contradictory SAR findings, such as the increase in hypocotyl diameter that coincided with the increase in hypocotyl length observed under PAR_{165}+FR_{35} for all species (Table 2). These findings could be a result of provoked SARs in the presence of FR light, while also providing a sufficient PPFD to promote regular modes of growth. Park and Runkle (2018) found that varying R:FR coupled with PPFD altered the intensity to which certain SARs occur, such as chlorophyll content and flowering occurrence in petunia (Park and Runkle 2018). Thus, while the inclusion of FR light did result in some SARs (e.g., increased hypocotyl length) in the present study, the sufficient PPFD provided to microgreens coinciding with the moderate R:FR resulted in few other shade symptoms observed (e.g., decreased hypocotyl diameter).

There are two possible reasons for the general increase in fresh and dry weight in PAR_{165}+FR_{35} compared to PAR_{165} (Table 2). As mentioned, leaf area increased for all three species under the FR abundant PAR_{165}+FR_{35}, potentially indicating further shade responses as plants attempt to maximize photon capture (Park et al. 2022). With microgreens under PAR_{165}+FR_{35} and PAR_{165} receiving the same PPFD based on PAR, the increase in leaf area

resulting from the inclusion of FR light in PAR_{165}+FR_{35} would improve photon capture and, as a result, increase biomass accumulation. The second reason for the increased fresh and dry weight under PAR_{165}+FR_{35} is the role of FR in driving photosynthetic activity via the excitation of PSI, resulting in the newly defined ePAR (Zhen and van Iersel 2017). In short, with PSI increasing in excitation from an abundance of FR light, both PSI and PSII can operate at a higher efficiency due to the two working at equilibrium, meaning the presence of FR light can increase the rate of photosynthesis (Park and Runkle 2018; Zhen and Bugbee 2020; Zhen and can Iersel 2017). In the present study, with FR wavelengths added to a predetermined adequate PPFD for microgreen production, there was no concern of PSII lacking in excitation. The role of FR light in driving photosynthetic activity for microgreens is further evidenced by the increase in both fresh and dry weight for mustard under PAR_{165}+FR_{35} compared to PAR_{200} (Table 2).

Full-mat results were similar to those for individual microgreens, with mustard and kohlrabi displaying similar fresh and dry weights between PAR_{200} and PAR_{165}+FR_{35} (Table 3). Additionally, red cabbage full-mat fresh and dry weights under PAR_{165}+FR_{35} increased compared to PAR_{200} (Table 3). Park and Runkle (2018) concluded from their experiments using FR light with petunia, that in the presence of a low R:FR, leaf growth was promoted when low R:FR was combined with an adequate PPFD (Park and Runkle 2018). Hence, the inclusion of supplemental FR light to lower the R:FR and contribute to photosynthesis is supported by the lack of difference (kohlrabi and mustard) or increase in biomass (red cabbage) between PAR_{165}+FR_{35} and PAR_{200}. However, since photosynthesis was not measured during this study, such conclusions require further research.

Microgreens grown under a temperature of 18 ºC had a slower rate of growth, inevitably resulting in reduced biomass compared to 21 ºC (Table 4). This trend continued for full-mat

fresh and dry weights for all species (Table 5). This is expected as plant growth and development decreases under a reduced air temperature (Kong et al. 2023). Still, multiple temperatures were evaluated to assess if a relationship between light and temperature was significant, and to assess how responses such as shade avoidance vary at different temperatures. Romero-Montepaone et al. (2020) concluded that SAR are more evident at warmer temperatures.

Through-trial measurements permitted a chronic visual check-in of trials, making progression pictures possible (Fig. 2-3). Microgreens grown at 21 ºC under PAR_{165}+FR_{35} surpassed the height of fully-grown microgreens (12 days) under PAR_{200} by day 7 (red cabbage; Fig. 1F) or day 9 (kohlrabi and mustard; Fig. 1B and 1D). While this is significant, visual aids further express the variation across environmental differences (Fig. 2-3). Visual analysis also sheds light on a visual variance, whereby microgreens grown at colder temperatures were stunted in growth but produced deeper coloration (presumed to be increased anthocyanins).

Concurrently, microgreens grown at warmer temperatures (21 ºC) under PAR_{165}+FR_{35} were seemingly ready for harvest prior to their planned destructive harvest 12 days after sowing. This visual insight also may permit a deeper understanding of how many days at a warm temperature under PAR_{165}+FR_{35} a microgreen needs to reach full size. As discussed previously, while microgreens grown under PAR_{165}+FR_{35} reached a desirable size (height) well before their harvest date, it possibly came at the cost of visually diluted pigmentation. Although phytochemical data was not recorded during this experiment. However, if a combination of far-red light (PAR_{165}+FR_{35}) and a warm air temperature (21 ºC) increases biomass or reduces production time, it may be plausible to explore means by which phytochemical concentrations reduced as a result of SAR may be reintroduced. One such example is a reduced air temperature, which has been shown to increase the accumulation of anthocyanins in plants (Boldt et al. 2014).

With the understanding that SARs may be impacted by temperature, we propose that warmer temperatures may be most conductive for beneficial microgreen characteristics achieved through the use of FR light. Nevertheless, through conducting this experiment at two temperatures, the downsides of SARs were exposed, as well as potential solutions. Since SAR's were emphasized at warmer temperatures to the point where harvest could occur earlier while pigmentation was visually reduced, there is potential for reducing the production time under FR light supplementation or replacement and transitioning to different environmental setting conducive to increased pigmentation (e.g., anthocyanins), such as a reduced air temperature. To best understand how a "dynamic" treatment may benefit microgreen production, an experiment in which microgreens are moved from a standard air temperature with a FR rich spectrum to a colder air temperature under the same spectrum on various days, may prove beneficial. Doing so could result in a production protocol through which both the benefits of temperature reduction (pigmentation) and FR light (SAR) occur.

Table 1. The mean ± *SD* of blue (B-PFD; 400-499 nm), green (G-PFD; 500-599 nm), red (R-PFD; 600-699 nm), far-red (FR-PFD; 700-750 nm), photosynthetically active radiation (PPFD; 400-700 nm), and extended photosynthetically active radiation (ePPFD; 400-750 nm) photon flux densities (PFD; $\mu mol \cdot m^{-2} \cdot s^{-1}$) as well as the ratio of red to far-red light (R:FR) for light treatments targeting a PPFD of 165 $\mu mol \cdot m^{-2} \cdot s^{-1}$ (PAR_{165}), a PPFD of 200 $\mu mol \cdot m^{-2} \cdot s^{-1}$ (PAR_{200}), or a PPFD of 165 $\mu mol \cdot m^{-2} \cdot s^{-1}$ plus 35 $\mu mol \cdot m^{-2} \cdot s^{-1}$ of far-red light (PAR_{165}+FR_{35}).

Light Treatment	B-PFD	G-PFD	R-PFD	FR-PFD	PPFD	ePPFD	R:FR
PAR_{165}	27.2 ± 0.8	66.0 ± 1.2	70.9 ± 1.4	1.6 ± 0.1	164.2 ± 1.7	165.8 ± 1.8	43.3 ± 2.4
PAR_{200}	34.1 ± 0.5	80.3 ± 1.0	86.4 ± 0.7	2.2 ± 0.2	200.7 ± 1.7	202.9 ± 1.8	39.9 ± 2.8
PAR_{165}+PAR_{35}	28.2 ± 0.4	66.3 ± 1.0	71.3 ± 1.0	36.2 ± 1.6	165.7 ± 2.4	201.9 ± 2.3	2.0 ± 0.1

Table 2. Main effect of light treatment on hypocotyl length, hypocotyl diameter, leaf number, leaf area, fresh weight, and dry weight for mustard (*Brassica juncea* 'Garnet Giant'), kohlrabi (*Brassica oleracea* var. *gongylodes*), and red cabbage (*Brassica oleracea* var. *capitata*) individual microgreens collected 12 d after sowing and grown under the following lighting treatments: photosynthetic photon flux density (PPFD) of 165 $\mu mol \cdot m^{-2} \cdot s^{-1}$ (PAR_{165}); PPFD of 200 $\mu mol \cdot m^{-2} \cdot s^{-1}$ (PAR_{200}); and PPFD of 165 $\mu mol \cdot m^{-2} \cdot s^{-1}$ + 35 $\mu mol \cdot m^{-2} \cdot s^{-1}$ of far-red light (PAR_{165}+FR_{35}).

Light Treatment	Length (mm)	Diameter (mm)	Leaf Number	Leaf Area (cm^2)	Fresh Weight (g)	Dry Weight (mg)
			Mustard			
PAR_{165}	33.8[i] a[ii]	1.37 a	3.6 a	2.14 a	0.11 a	5.8 a
PAR_{200}	35.1 a	1.45 a	3.6 a	2.55 a	0.12 a	6.9 a
PAR_{165}+FR_{35}	46.9 b	1.58 b	4.1 b	4.28 b	0.17 b	12.5 b
			Kohlrabi			
PAR_{165}	29.5 a	1.22 a	2.2	2.16 a	0.10 a	7.6 a
PAR_{200}	32.7 a	1.32 ab	2.3	2.65 ab	0.16 b	10.2 b
PAR_{165}+FR_{35}	49.9 b	1.41 b	2.2	3.11 b	0.15 b	11.7 b
			Red Cabbage			
PAR_{165}	17.6 a	1.34 a	2.4	2.75 a	0.11 a	9.4
PAR_{200}	17.6 a	1.32 a	2.4	2.87 ab	0.13 ab	10.9
PAR_{165}+FR_{35}	34.1 b	1.48 b	2.4	3.50 b	0.15 b	12.1

[i] Mean values are based on five individual microgreens from each treatment replication, with six total replications across two experimental repetitions.

[ii] Means sharing a letter are not statistically different by Tukey's honestly significant difference (HSD) test at $P \leq 0.05$. Means with no lettering were found to have no significant difference between treatments.

Table 3. Main effect of light treatment on the fresh weight and dry weight for mustard (*Brassica juncea* 'Garnet Giant'), kohlrabi (*Brassica oleracea* var. *gongylodes*), and red cabbage (*Brassica oleracea* var. *capitata*) microgreen mats collected 12 d after sowing and grown under the following lighting treatments: photosynthetic photon flux density (PPFD) of 165 $\mu mol \cdot m^{-2} \cdot s^{-1}$ (PAR_{165}); PPFD of 200 $\mu mol \cdot m^{-2} \cdot s^{-1}$ (PAR_{200}); and PPFD of 165 $\mu mol \cdot m^{-2} \cdot s^{-1}$ + 35 $\mu mol \cdot m^{-2} \cdot s^{-1}$ of far-red light (PAR_{165}+FR_{35}).

Light Treatment	Fresh Weight (g)	Dry Weight (g)
	Mustard	
PAR_{165}	73.9[i]	4.13
PAR_{200}	78.4	4.27
PAR_{165}+FR_{35}	70.6	4.09
	Kohlrabi	
PAR_{165}	40.6 a[ii]	3.35 a
PAR_{200}	52.8 b	4.14 b
PAR_{165}+FR_{35}	49.7 b	3.82 b
	Red Cabbage	
PAR_{165}	18.4 a	1.64 a
PAR_{200}	21.1 a	1.77 ab
PAR_{165}+FR_{35}	26.8 b	1.90 b

[i] Mean values are based on three total replications across one experimental repetition.

[ii] Means sharing a letter are not statistically different by Tukey's honestly significant difference (HSD) test at $P \leq 0.05$. Means with no lettering were found to have no significant difference between treatments.

Table 4. Main effect of the temperature treatment on hypocotyl length, hypocotyl diameter, leaf number, leaf area, fresh weight, and dry weight for mustard (*Brassica juncea* 'Garnet Giant'), kohlrabi (*Brassica oleracea* var. *gongylodes*), and red cabbage (*Brassica oleracea* var. *capitata*) individual microgreens collected 12 d after sowing and grown under an ambient air temperature of either 18 or 21 °C.

Temperature Treatment	Length (mm)	Diameter (mm)	Leaf Number	Leaf Area (cm^2)	Fresh Weight (g)	Dry Weight (mg)
			Mustard			
18 °C	32.7[i] a[ii]	1.38 a	3.5 a	2.33 a	0.10 a	6.7 a
21 °C	44.5 b	1.56 b	3.9 b	3.65 b	0.17 b	10.1 b
			Kohlrabi			
18 °C	31.4 a	1.21 a	2.0 a	2.15 a	0.11 a	8.5 a
21 °C	43.4 b	1.43 b	2.5 b	3.13 b	0.16 b	11.2 b
			Red Cabbage			
18 °C	21.1 a	1.29 a	2.1 a	2.59 a	0.10 a	9.6 a
21 °C	25.1 b	1.47 b	2.7 b	3.49 b	0.15 b	12.0 b

[i] Mean values are based on five individual microgreens from each treatment replication, with six total replications across two experimental repetitions.

[ii] Means sharing a letter are not statistically different by Tukey's honestly significant difference (HSD) test at $P \leq 0.05$.

Table 5. Main effect of the temperature treatment on the fresh weight and dry weight for mustard (*Brassica juncea* 'Garnet Giant'), kohlrabi (*Brassica oleracea* var. *gongylodes*), and red cabbage (*Brassica oleracea* var. *capitata*) microgreens mats collected 12 d after sowing and grown under an ambient air temperature of either 18 or 21 °C.

Temperature Treatment	Fresh Weight (g)	Dry Weight (g)
	Mustard	
18 °C	48.3[i] a[ii]	3.60 a
21 °C	100.3 b	4.73 b
	Kohlrabi	
18 °C	29.3 a	3.30 a
21 °C	66.1 b	4.24 b
	Red Cabbage	
18 °C	11.3 a	1.22 a
21 °C	32.9 b	2.32 b

[i] Mean values are based on three total replications across one experimental repetition.

[ii] Means sharing a letter are not statistically different by Tukey's honestly significant difference (HSD) test at $P \leq 0.05$. Means with no lettering were found to have no significant difference between treatments.

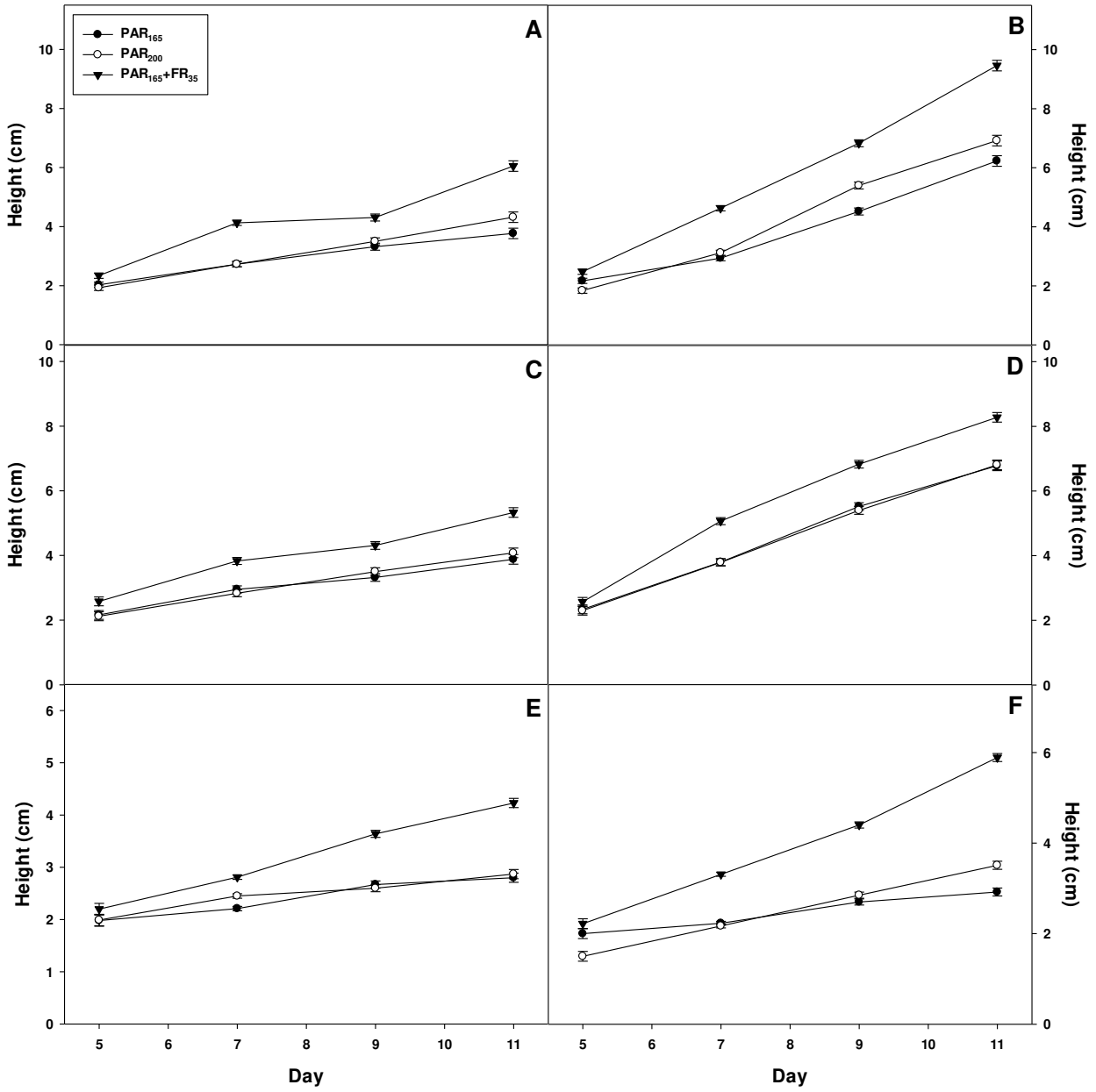

Figure 1. Mean ± standard error of non-destructive height collected every two days, beginning five days after sowing, for kohlrabi (A, B; *Brassica oleracea* var. *gongylodes*), mustard (C, D; *Brassica juncea* 'Garnet Giant'), and red cabbage (E, F; *Brassica oleracea* var. *capitata*) microgreen mats grown under an ambient air temperature of either 18 (A, C, E) or 21 ºC (B, D, F) and subjected to the following lighting treatments: photosynthetic photon flux density (PPFD) of 165 $\mu mol \cdot m^{-2} \cdot s^{-1}$ (PAR_{165}); PPFD of 200 $\mu mol \cdot m^{-2} \cdot s^{-1}$ (PAR_{200}); and PPFD of 165 $\mu mol \cdot m^{-2} \cdot s^{-1}$ + 35 $\mu mol \cdot m^{-2} \cdot s^{-1}$ of far-red light (PAR_{165}+FR_{35}).

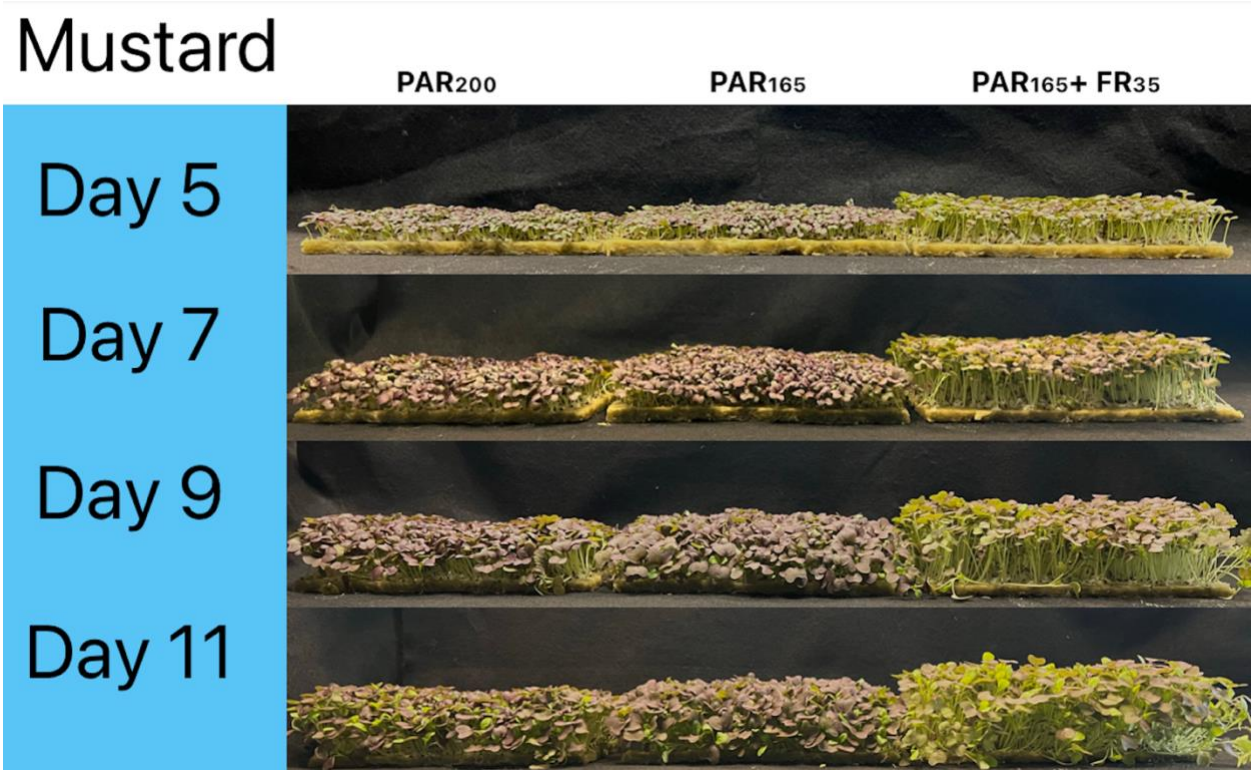

Figure 2. Photographs collected every two days, beginning five days after sowing, for mustard (*Brassica juncea* 'Garnet Giant') microgreen mats grown under an ambient air temperature of 21 ºC and subjected to the following lighting treatments: photosynthetic photon flux density (PPFD) of 165 µmol·m^{-2}·s^{-1} (PAR_{165}); PPFD of 200 µmol·m^{-2}·s^{-1} (PAR_{200}); and PPFD of 165 µmol·m^{-2}·s^{-1} + 35 µmol·m^{-2}·s^{-1} of far-red light (PAR_{165}+FR_{35}).

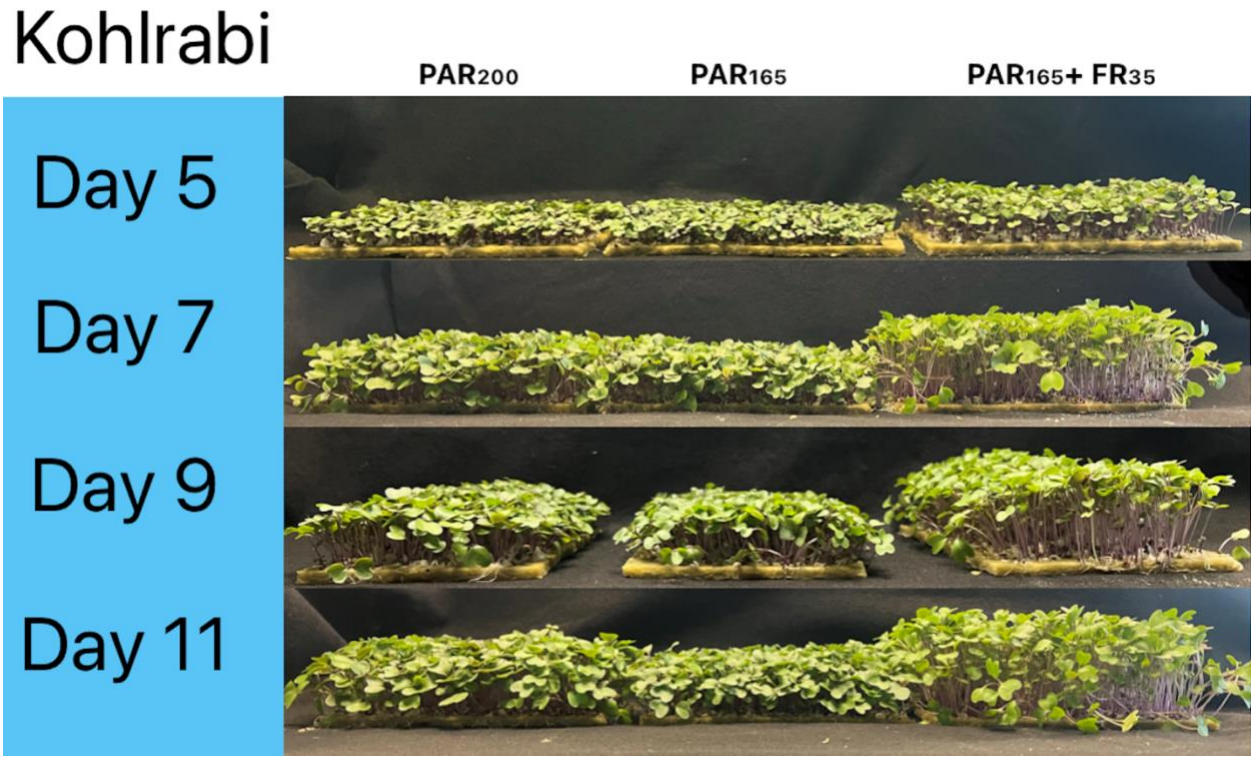

Figure 3. Photographs collected every two days, beginning five days after sowing, for kohlrabi (*Brassica oleracea* var. *gongylodes*) microgreen mats grown under an ambient air temperature of 21 ºC and subjected to the following lighting treatments: photosynthetic photon flux density (PPFD) of 165 µmol·m^{-2}·s^{-1} (PAR_{165}); PPFD of 200 µmol·m^{-2}·s^{-1} (PAR_{200}); and PPFD of 165 µmol·m^{-2}·s^{-1} + 35 µmol·m^{-2}·s^{-1} of far-red light (PAR_{165}+FR_{35}).

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CHAPTER 3. THE EFFECT OF REDUCED AIR TEMPERATURE DURATION ON THE MORPHOLOGY AND QUALITY OF RED CABBAGE MICROGREENS GROWN IN A CONROLLED ENVIRONMENT

3.1 Introduction

Temperature adjustments during production are often viewed seasonally or through day/night cycles rather than a dynamic shift throughout production. Microgreens are a food crop promoted and advertised for their density of various nutrients beneficial to human health. Nutrients include micronutrients such as iron and zinc, and phytonutrients like anthocyanins (Verlinden 2019). Microgreens require minimal production inputs (Kyriacou et al. 2016) and have a relatively short production duration, being harvested 7 to 14 days after sowing. The saleable product for microgreens consists of a hypocotyl, cotyledons, and one to two true leaves (between the sprout and baby greens phases of growth) (Choe et al. 2018). Species in the genus *Brassica* are often used for microgreen production and research (Liu et al. 2022; Samuolienė et al. 2013). As mentioned, microgreen production inputs are minimal (Kong et al. 2023). For example, little to no fertilizer is recommended as common production practice (Moraru et al. 2022). Additionally, a low photosynthetic photon flux density (PPFD; $\mu mol \cdot m^{-2} \cdot s^{-1}$) is generally used for commercial production (Jones-Baumgardt et al. 2019; Kong et al. 2023). Previous research has shown that a low PPFD comprised of photosynthetically active radiation (PAR; 400-700 nm) (Kusuma and Bugbee 2023; Zhen et al. 2021) is sufficient for the production of microgreens, with a saturation in terms of yield and quality benefits occurring around 300 $\mu mol \cdot m^{-2} \cdot s^{-1}$ (Jones-Baumgardt et al. 2019). This was understood by comparing *Brassica* microgreens grown under different PPFDs of red (600-700 nm) and blue (400-500 nm) light to create PPFDs of 100, 200, 300, 400, 500, and 600 $\mu mol \cdot m^{-2} \cdot s^{-1}$ (Jones-Baumgardt et al. 2019).

In doing so, it was found that increasing PPFD coincided with increasing fresh weight up to 300 and 400 $\mu mol \cdot m^{-2} \cdot s^{-1}$, after which benefits to yield saturated with further increases in PPFD (Jones-Baumgardt et al. 2019).

With the introduction of light-emitting diodes (LED) for horticultural crop production, manipulation of both light spectrum and intensity allows for desired lighting targets to be achieved (Morrow 2008) and further implications of light, such as interactions with other environmental factors, can be explored. LEDs are also beneficial due to their minimal emission of radiant heat, high electrical efficacy, and potential for dimming and spectral manipulation (Morrow 2008). In our experiment in Chapter 2, possible interactions between light and temperature for the production of microgreens were explored, while spectral quality and quantity remained constant, with the inclusion of far-red (FR) light (700-780 nm). This research was executed to understand the importance of FR light in both photomorphology and photochemistry for microgreens grown at two ambient air temperatures. While trends of a possible interaction between FR light and temperature follow similar conclusions by Jeong et al. (2024) regarding shade avoidance, there was emphasis placed on the visual quality of microgreens when stress responses were provoked (e.g., reduced air temperature) in our previous experiment. Kong et al. (2023) found that FR light also impacts pigmentation for mustard (*Brassica juncea*) microgreens under varying temperatures. Lanoue et al. (2022) concluded that phenolic concentrations of microgreens were maintained or increased under a low PPFD with a 24-h photoperiod compared to a 16-h photoperiod. Liu et al. (2022) also found an increase in soluble protein content for cabbage (*Brassica oleracea*) microgreens at a PPFD of 90 $\mu mol \cdot m^{-2} \cdot s^{-1}$ compared to lower PPFD values ranging down to 30 $\mu mol \cdot m^{-2} \cdot s^{-1}$. Therefore, it is clear that both temperature and light play vital roles in the development of pigmentation for microgreens.

The photosynthetic impacts of FR light are discussed in detail by Zhen et al. (2022), resulting in a new definition of extended PAR (ePAR; 400-750 nm). Particularly, FR wavelengths ranging from 700-750 nm are understood to have a photosynthetic effect in combination with current wavelengths defined as PAR (Kusuma and Bugbee 2023; Zhen et al. 2021). Far-red light is abundant in shade, where it causes plants to express elongated characteristics in such low light conditions. These characteristics are referred to as shade avoidance responses (SAR) (Smith and Whitelam 1997) and include increased stem or petiole elongation, increased or inhibited leaf expansion, and potential reduced pigmentation (Gerovac et al. 2016; Kong et al. 2023; Wang et al. 2015). While SARs are comprehensively understood and recorded, strategies to benefit horticultural production are limited.

Jeong et al. (2024) concluded that there is an interaction between light and temperature when regulating plant morphology, found when comparing lettuce *(Lactuca sativa*) grown with a 0%, 10% or 20% presence of FR in experimental spectra across warm and cold temperatures. It was concluded that FR in combination with colder temperatures enhanced lettuce leaf expansion and yield (Jeong et al. 2024). Additionally, arugula microgreens grown at an air temperature of 28 ºC under monochromatic blue (450 nm peak) or red (670 nm peak) LED sole-source lighting had increased hypocotyl elongation of 37% under blue compared to red light at a PPFD of 110 $\mu mol \cdot m^{-2} \cdot s^{-1}$ (Kong et al. 2023). While it is generally understood that plants growth and development increases at warmer temperatures up to an optimum, there is a need to further understand how SAR may interact with temperature.

Dynamic variable treatments for horticultural production are not uncommon, but temperature changes are often associated with time of day, and not point-in-time for production. For example, Shibaeva et al. (2018) explored the impacts of a diurnal temperature drop during

young cucumber (*Cucumis sativus*) production. This study looked at the impact of temperature reduction within a day span, concluding that reducing the production temperature was only effective if applied at the end of the night (Shibaeva et al. 2018). Much of this dynamic temperature work was an extension of previous temperature alteration work done by Xiong et al. (2002), which altered daytime (DT) and nighttime temperatures (NT) to create DIF treatments. DIF is an acronym used to characterize the difference in DT and NT, resulting in either a positive, negative, or zero value (Ballaré and Pierik 2017). A positive DIF (+DIF) is associated with promoting growth in ways such as increased plant tissue elongation. On the contrary, a negative DIF (-DIF), with higher NT and lower DT, suppresses plant tissue elongation (Ballaré and Pierik 2017; Kong et al. 2023). Findings from Xiong et al. (2002) followed suit with aspects of +DIF and –DIF, but discovered that supplementing plants with FR light at the end of the day (EOD-FR) resulted in SAR characteristics such as increased elongation, reducing the negative effects of –DIF. There is a clear need to better define dynamic temperature treatments, to determine whether DIF or potentially other modes of temperature alteration during production may be horticulturally beneficial.

To date, there is limited information on how temperature alterations with the inclusion of FR light in sole-source light spectra impacts plant growth and morphology. The objective of this study was to investigate the benefit of moving red cabbage (*Brassica oleracea* var. *capitata*) microgreens grown at an air temperature of 21 ºC to a controlled environment with an air temperature of 16 ºC. The categorical separation defining each treatment is the number of days spent in the warm environment of 21 ºC, before moving into the cold environment of 16 ºC. We hypothesized that the inclusion of FR light in the light spectrum would result in characteristic SAR (e.g., increased hypocotyl elongation, leaf area, etc.) regardless of temperature, and a shift

to a lower air temperature would serve as a secondary stressor to increase phytochemical concentrations (e.g., anthocyanins) as a means of counteracting a potential decrease in pigmentation resulting from the FR light induced SAR.

3.2 Materials and Methods

3.2.1 Plant Material, Culture, and Germination

Seeds of red cabbage (*Brassica oleracea* var. *capitata*) were sown on 16.5 x 25.4 cm (419.4 cm^2) sections of rockwool soilless substrate (Grodan Rockwool Micro-Greens Cress Propagation Mat; Milton, Ontario, CA) with a seed density of 3.5 g per rockwool section. Rockwool mats were cut and soaked in tap water prior to sowing, and immediately placed in walk-in growth chambers (GR64; Conviron, Winnipeg, Canada).

Sections were placed in trays (52 × 26 × 6 cm) without drainage holes, along with one cup of water-soluble fertilizer (pH: 5.5-6.5, EC: 0.75 $mS \cdot cm^{-1}$) using 13N–0.9P–10.8K Plug Special (Plant Marvel; Chicago Heights, IL) providing (in $mg \cdot L^{-1}$) 150 nitrogen (N), 23 phosphorus (P), 150 potassium (K), 69 calcium (Ca), 34 magnesium (Mg), 0.15 boron (B), 0.07 copper (Cu), 0.75 iron (Fe), 0.37 manganese (Mn), 0.07 molybdenum (Mo), and 0.37 zinc (Zn). Trays were covered with humidity domes until the completion of the four-day germination period, in total darkness. This was done at an air temperature and relative humidity setpoint of 24 ºC and 55%/65% (D/N) respectively.

3.2.2 Growth Chamber Environment

Four days after sowing, humidity domes were removed, and sections transferred to webbed trays (52 × 26 × 6 cm). Webbed trays were then placed in ebb and flow tables and irrigated every 3 hours using the same fertilizer described above with a target EC of 0.75 $mS \cdot cm^{-1}$. A target pH of 5.5 to 6.0 was maintained by adding phosphoric acid (pH down;

General Hydroponics, Santa Rosa, CA). pH and EC were measured using a handheld probe (Growline H19814; Hanna Instruments, Woonsocket, RI) and system adjustments were made as needed every two days.

Two growth chambers were maintained at an air temperature setpoint of either 16 or 21 ^{o}C, with temperature averages ± standard deviation of 16.7 ± 0.5 ºC and 21.3 ± 2.0 ºC for the duration of the experiment. Relative humidity averages ± standard deviation for the air temperature setpoints of 16 and 21 ^{o}C were 60.3% ± 7.0% and 73.6% ± 9.0%, respectively. Temperature and relative data was collected using external temperature meters (HOBO UX100-011A; Onset, Bourne, MA). Each growth chamber consisted of four counterbalanced light canopies (RAY44 Physiospec Indoor and Far-red fixtures; Fluence, Austin TX), of which three were used per trial. A PPFD of 165 $\mu mol \cdot m^{-2} \cdot s^{-1}$ + 35 $\mu mol \cdot m^{-2} \cdot s^{-1}$ of FR light (PAR_{165}+FR_{35}) was used under each of the three light bays. Light contamination was reduced due to homogenous lighting; nevertheless, shade curtains were used to increase evenness under each light bay.

To achieve environmental homogeneity with sole-source lighting, light must be measured at its point of incidence across a plane to create a homogenous light plane. This is done so by adjusting flux of the lights via an output percentage, as well as the physical distance of the lights to the plant. The light setting was established at the beginning of each trial using a spectrometer (LI-180 Spectrometer, Li-COR Biosciences, Lincoln, NE), with average ± SD of various light parameters characterizing each treatment (Table 6). To further ensure uniform light distribution under each light canopy, trays were rotated every other day.

Two walk-in growth chambers (GR64; Conviron) were used for this study, of which one served both for the germination (24 ºC) and warm temperature of 21 ºC while the other served as

the cold environment shift to 16 ºC. The warm and cold chambers would alternate after each experiment to control for chamber effects. On day 6, 8, 10, and 11, trays (52 × 26 × 6 cm) containing two microgreen mat sections each were transferred from the warm (21 ºC) chamber into the cold (16 ºC) chamber. One grouping of microgreens was left at 21 ºC for the entire experiment to serve as a control; thus, a total of five temperature treatments were evaluated for this experiment. A summary of the five temperature treatments (Table 7).

3.2.3 Microgreen Data Collection

Destructive data for individual microgreens and full mats were collected on day 12. For individual plant microgreen data, five microgreens were selected at random from each mat section and data was collected on fresh weight (g) using a balance (Analytical Balance ME54E, Mettler Toledo Ltd, Columbus, OH), hypocotyl length (cm), hypocotyl diameter (mm) using a caliper (*Fisherbrand* Traceable; Digital Calipers, Waltham, MA) width (mm), leaf count, and leaf area (cm^2; Li-COR LI3100C Area Meter, Li-COR Biosciences, Lincoln, NE). Microgreens were then dried at 70 °C for at least five days to determine the dry weight (mg) of each using an analytical microbalance (Analytical Balance ME54E; Mettler Toledo Ltd). A handheld multiple pigment meter (Optisciences MPM-100) was used to measure the top and bottom of one cotyledon per individual microgreen. Fresh and dry weight data were also taken on the remaining microgreens for each mat, using the same drying protocol described above.

3.2.4 Statistical Analysis

Experimental trials were conducted between September 26, 2023, and October 30, 2023, at the CSU Spur Campus in Denver, Colorado. This experiment was a randomized complete block design with dynamic temperature treatments (5 levels) as treatment factors and repetition (2 levels) as a blocking variable. The blocking variable (repetition) was included in the analysis,

with the temperature conditions within each chamber switched between repetitions. Destructive data analysis was comprised of three microgreen mats per repetition, resulting in a total of six replications for the experiment. Every "Day" variable was compared to one another to assess the difference in change over time. The number following "Day" represents the day after sowing during production in which the microgreens mats were moved from the warm (21 ºC) environment to the cold (16 ºC) environment. Analysis was conducted using a pairwise comparison, constructed from estimated marginal means individually created for "Day". This was concluded using Tukey's honestly significant difference (HSD) test at $P \leq 0.05$, and a type III ANOVA was conducted using R statistical software (Lenth, 2021; R Core Team, 2021).

3.3 Results

3.3.1 Hypocotyl Length and Hypocotyl Diameter

Day 6 and Day 8 microgreens, the two treatments with the longest duration at 16 ºC, had shorter hypocotyls than Day 10, Day 11, and Control (Table 8). For example, Control microgreens had 33% longer hypocotyls than Day 6 (Table 8). The latter grouping of Day 10, Day 11, and Control microgreens had similar hypocotyl lengths (Table 8). Day 8 microgreens resulted in similar hypocotyl diameter to all treatments, of which Day 6 was smaller than Day 10, Day 11, and Control (Table 8). The difference in hypocotyl diameter between microgreens with the longest duration in cold (Day 6) and microgreens that received no time in a cold environment (Control) was not significant, with an increase of only 7.9% (Table 8).

3.3.2 Leaf Count and Leaf Area

No differences in leaf number were observed for any of the temperature treatments (Table 8). Day 11 microgreens had the largest leaf area, which was found to be greater than

Control but similar to Day 10 (Table 8). For example, leaf area of Day 11 microgreens was 24% greater than Control (Table 8). Longer durations at the 16 ºC temperature treatment resulted in reduced leaf expansion, as leaf area was 33% smaller for Day 6 microgreens compared to Day 11 (Table 8). Relative anthocyanin content for both the adaxial and abaxial surface of cotyledons was similar for all treatments (Table 9).

3.3.3 Fresh and Dry Weight

No difference in fresh or dry weight between Day 6 and Day 8 were observed, but fresh weight for both treatments was smaller compared to Day 10, Day 11, and Control. Dry weight for Day 6 and Day 8 was also smaller compared to Day 10 and Day 11, but similar to Control (Table 8). Concurrently, there was no difference in fresh weight between Day 10, Day 11, and Control microgreens (Table 8). While no difference in fresh weight was observed between Day 10, Day 11, and Control, dry weight was 28% greater for Day 10 microgreens compared to Control (Table 8).

The full mat microgreen analysis followed the same trend as the individual microgreens for fresh weight, with Day 6 and Day 8 microgreens being similar and, generally, smaller than Day 10, Day 11, and Control (Table 10). The largest fresh and dry weight was observed for Day 10 microgreens, although differences with Day 11 and Control were not observed (Table 10).

3.4 Discussion

Findings from Chapter 2 were used to cultivate a follow-up experiment on the light treatment PAR_{165}+FR_{35}, with dynamic temperature treatments. With the inclusion of FR light in sole-source lighting for microgreen production as a constant, the alteration of temperature throughout production provides an opportunity to observe impacts on both SAR and microgreen

quality attributes (e.g., pigmentation). Far-red light is understood to decrease anthocyanin content in lettuce, but this response is species-specific (Liu and van Iersel 2022). Thus, not only could a decrease in air temperature be beneficial for controlling SAR in controlled environments, but also provides a possible opportunity to alter pigmentation for red cabbage microgreens (Boldt et al. 2014; Liu and van Iersel 2022).

Red cabbage microgreens grown for at least 4 days at 16 ºC (Day 6 and Day 8) were smaller in hypocotyl length, leaf area, and fresh and dry weight compared to the other temperature treatments (Table 8). Smaller microgreens produced under this reduced temperature for longer durations may simply be due to a well-documented suppression of growth at lower temperatures (Romero-Montepaone et al. 2020). Warmer temperatures allowed for typical SAR in response to FR light to occur, and possibly even emphasized these responses as documented by Romero-Montepaone et al. (2020). The increase in growth characterized by increased hypocotyl length, leaf area, and biomass from Control, Day 10, and Day 11 microgreens in comparison to Day 6 and Day 8 is a potential indicator of beneficial SAR from both a constant abundance of FR light and warmer temperatures. The increase in leaf area and dry weight observed for Day 11 compared to Control microgreens indicates a possible synergistic interaction between FR light and temperature (Table 8). This response could be due to a species-dependent reaction to temperature extremes (Hatfield and Prueger 2015), or possibly a species-specific response to FR light under reduced temperatures (Patel et al. 2013). For example, Patel et al. (2013) found that leaf area responses in Arabidopsis (*Arabidopsis thaliana*) were dependent on temperature, with Arabidopsis displaying an increase in leaf area under a low red to FR light ratio (R:FR) at a reduced air temperature of 16 compared to 22 ºC. Phytochrome B (Phy_B) is also understood to be regulated by temperature (Jeong et al. 2024), converting back to P_r at a rate

dependent on air temperature (Ballaré and Pierik 2017; Thingnaes et al. 2008). Still, why a single day under a reduced air temperature (Day 11) would result in increased leaf area and dry weight compared to Control microgreens requires further research.

Similarly, the trend of higher full mat fresh and dry weight on Day 10 and Day 11 compared to Control microgreens requires further investigation (Table 10). However, this response could be indicative of an optimal dynamic temperature combination to achieve microgreens that are both large and phytochemically dense. There may also be a need to explore dynamic temperature treatments on red cabbage microgreens that are given a longer time to develop before receiving an air temperature drop. For example, better indicator for reduced temperatures may be the emergence of the first true leaf, rather than a pre-determined time within a 12-day production period. Further, exploring such phenomena may be more effective with more mature plants, such as baby greens, which are grown and harvested for the consumption of true leaves.

While results were not significant, there was a distinct trend of increasing relative anthocyanin content under longer durations of the cold temperature (Table 9). This trend was present on measurements collected for both the abaxial and adaxial surface of the cotyledons. It is understood that plants produce anthocyanins as a stress response to colder temperatures (Boldt et al. 2014). For example, in a recent study introducing the normalized difference anthocyanin index (NDAI) as a low-cost tool for assessing anthocyanin content, Kim and van Iersel (2023) utilized air temperatures of 4, 12, and 20 °C for durations of 0, 12, 24, or 36 hours to induce a range of anthocyanin concentrations in red-leaf lettuce (*Lactuca sativa*) cultivars ‘Rouxai’ and ‘Teodore’. However, while short durations of cold temperature have been shown to increase anthocyanin concentrations, further research is needed to assess the reduced air temperature

setpoint, timing, and duration to optimize anthocyanin concentrations for red cabbage microgreens based on the current study.

Red cabbage microgreens grown at 21 ºC until the final day of production, then placed in a cold environment until harvest may prove to be a beneficial method of increasing the microgreen nutrition and visual quality. Approaching temperature as a dynamic input enables potential new methodologies of horticultural production. For example, microgreens could be sold unharvested without a negative effect to their quality and stored in a refrigerated setting. However, to facilitate such methodology, further research regarding the relationship between FR light and temperature is necessary.

Table 6. Average ± standard deviation of blue (B-PFD; 400-499 nm), green (G-PFD; 500-599 nm), red (R-PFD; 600-699 nm), far-red (FR-PFD; 700-750 nm), photosynthetic (PPFD; 600-700 nm), and extended photosynthetic (ePPFD; 400-750 nm) photon flux densities (PFD; $\mu mol \cdot m^{-2} \cdot s^{-1}$) as well as the red: far-red ratio (R:FR) of the growth chamber lighting targeting a PPFD of 165 $\mu mol \cdot m^{-2} \cdot s^{-1}$ plus 35 $\mu mol \cdot m^{-2} \cdot s^{-1}$ of far-red light (PAR_{165}+FR_{35}).

Light Treatment	B-PFD	G-PFD	R-PFD	FR-PFD	PPFD	ePPFD	R:FR
PAR_{165}+PAR_{35}	27.3 ± 1.03	66.5 ± 2.51	71.2 ± 2.59	34.5 ± 2.55	165.1 ± 6.1	199.5 ± 7.07	1.9 ± 0.15

Table 7. Description of air temperature setpoint durations for dynamic temperature treatments.

Temperature Treatment	Germination 24 ºC	Warm 21 ºC	Cold 18 ºC
Day 6	4	2	6
Day 8	4	4	4
Day 10	4	6	2
Day 11	4	7	1
Control	4	8	0

Table 8. Effect of reduced air temperature duration on hypocotyl length, hypocotyl diameter, leaf number, leaf area, fresh weight, and dry weight for red cabbage (*Brassica oleracea* var. *capitata*) individual microgreens collected 12 d after sowing.

Temperature Treatment	Length (mm)	Diameter (mm)	Leaf Number	Leaf Area (cm^2)	Fresh Weight (g)	Dry Weight (mg)
Day 6	29.6[i] a[ii]	1.25 a	2.03	2.02 a	0.09 a	7.46 a
Day 8	31.0 a	1.34 ab	2.03	2.17 ab	0.10 a	8.10 a
Day 10	38.6 b	1.39 b	2.03	2.73 cd	0.12 b	10.39 bc
Day 11	38.7 b	1.44 b	2.03	3.01 d	0.13 b	11.02 c
Control	39.4 b	1.40 b	2.10	2.43 bc	0.12 b	8.61 ab

[i] Mean values are based on five individual microgreens from each treatment replication, with six total replications across two experimental repetitions.

[ii] Means sharing a letter are not statistically different by Tukey's honestly significant difference (HSD) test at $P \leq 0.05$. Means with no lettering were found to have no significant difference between treatments.

Table 9. Effect of reduced air temperature duration on relative anthocyanin content of both the abaxial and surface of cotyledons for red cabbage (*Brassica oleracea* var. *capitata*) individual microgreens collected 12 d after sowing.

Temperature Treatment	Relative Anthocyanin (Adaxial)	Relative Anthocyanin (Abaxial)
Day 6	0.06[i]	0.06
Day 8	0.05	0.05
Dy 10	0.04	0.04
Day 11	0.03	0.03
Control	0.03	0.03

[i] Mean values are based on six total replications across two experimental repetitions.
[ii] Means with no lettering were found to have no significant difference between treatments by Tukey's honestly significant difference (HSD) test at $P \leq 0.05$

Table 10. Effect of reduced air temperature duration on the fresh and weight of red cabbage (*Brassica oleracea* var. *capitata*) microgreens mats collected 12 d after sowing.

Temperature Treatment	Fresh Weight (g)	Dry Weight (g)
Day 6	14.4 [i] a[ii]	1.21 a
Day 8	15.9 a	1.29 ab
Dy 10	24.4 b	1.84 c
Day 11	23.0 b	1.70 c
Control	23.4 b	1.56 bc

[i] Mean values are based on five individual microgreens from each treatment replication, with six total replications across two experimental repetitions.
[ii] Means sharing a letter are not statistically different by Tukey's honestly significant difference (HSD) test at $P \leq 0.05$.

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