Exploration on Using Light-Emitting Diode Spectra to Improve the Quality and Yield of Microgreens in Controlled Environments

by

Qinglu Ying

A Thesis

presented to

The University of Guelph

In partial fulfilment of requirements
for the degree of

Doctor of Philosophy

in

Environmental Sciences

Guelph, Ontario, Canada

ABSTRACT

EXPLORATION ON USING LIGHT-EMITTING DIODE SPECTRA TO IMPROVE THE QUALITY AND YIELD OF MICROGREENS IN CONTROLLED ENVIRONMENTS

Qinglu Ying
University of Guelph, 2020

Advisors:
Dr. Youbin Zheng

Light is essential for plant production, and adjusting light quality has potential in enhancing their desired growth characteristics and secondary metabolite profiles. This study explored the effects of light spectral quality from light-emitting diodes (LEDs) on the growth, yield, appearance quality and phytochemical content in four species of *Brassicaceae* microgreens (arugula, cabbage, kale and mustard) in controlled environments. Microgreens were cultivated under a combination of blue (B) and red (R) LEDs with different photon flux ratios from B_5R_{95} to $B_{30}R_{70}$ at photosynthetic photon flux density of 300 $\mu mol \cdot m^{-2} \cdot s^{-1}$ with 16-hour photoperiod. The fresh weight (FW) and dry weight of microgreens were generally not affected, except for cabbage. However, the hypocotyl length and cotyledon area of kale and mustard decreased proportionally as B light percentage increased. Moreover, the hue angle of cotyledon color decreased when B light percentages increased in four microgreen species. Under the same lighting treatments, varying B and R ratio did not affect the extractable levels of total chlorophyll, carotenoid, or nitrate content in any of the four microgreen species. Conversely, increasing B light percentage elevated ascorbate, total phenolic and total anthocyanin levels, although the magnitude of the elevation varied across species and phytochemicals. To increase the height of microgreens for facilitating machine harvest, low intensity supplemental lighting

strategies supplied at night were evaluated in both growth chamber and greenhouse. In the growth chamber, 20 $\mu mol \cdot m^{-2} \cdot s^{-1}$ supplemental B light with or without 20 $\mu mol \cdot m^{-2} \cdot s^{-1}$ far-red light for 8-hour during the night effectively promoted stem elongation without compromising microgreen visual quality or yield. The plants were grown under 300 $\mu mol \cdot m^{-2} \cdot s^{-1}$ $B_{20}R_{80}$ LED lighting for 16 hours during the day. Similarly, supplementing 14 $\mu mol \cdot m^{-2} \cdot s^{-1}$ B light at night promoted stem elongation by 10% and 16% in arugula and mustard microgreens, respectively, in the greenhouse during winter months; this coincided with respective 32% and 29% increase in FW. Total chlorophyll, carotenoid, and phenolic levels were unaffected by supplemental B light. This thesis concludes that light quality significantly affects plant morphology and secondary metabolism, and provides useful information for commercial growers to improve their production using cost-efficient lighting strategies.

ACKNOWLEDGEMENTS

To start with, I would like to sincerely thank my advisor, Dr. Youbin Zheng for giving me such a great opportunity to learn and offering me guidance whenever I need it. You were always available whenever I went into trouble or needed advice. I am grateful to have you as my supervisor, and I have learnt so many things from you for both academia and life. I would also like to thank Dr. Gale Bozzo and Dr. Mike Dixon for serving as my advisory committee and sharing their invaluable knowledge and advices on experiment design, analyses and scientific writing.

I would like to thank Natural Sciences and Engineering Research Council of Canada (NSERC) and our industrial partner Greenbelt Microgreens, for their financial support. Ian Adamson and Alice Ferris, I cannot thank you enough for always willing to answer all the questions I have and providing the experimental materials for my study.

Also, a special thank you to Dr. Yun Kong and David Llewellyn for your time and effort to make my experiments happened and providing valuable insight on my papers. I have learnt to think critically and creatively as a researcher, which will definitely help me in my future life and career. Thank you to everyone in Zheng's lab, Chase Jones-Baumgardt, Katie Schiestel, Deron Caplan, Jasmine Mah, Mitchell Eicher-Sodo, Devdutt Kamath, Melissa Moher, Brandon Yep, Lewys Bevan and Victoria Rodriguez Morrison. Thank you all for providing useful thoughts and sharing knowledge when I had questions about my experiments and writing. Special thank to Chase, I will never forget the two-year time that we spent together building the growth chamber, harvesting and doing phytochemical analyses. Thank you so much for always supporting me and making me laugh.

Last but not the least, I would also like to say thank you to my family, my grandma, mom and dad, for always supporting me and encouraging me all the time. Thank you to all my friends in Canada and China, especially Alvin for accompanying me and believing in me.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF SYMBOLS, ABBREVIATIONS OR NOMENCLATURE

ALA – Aminolevulinic acid

B – Blue

DAS – Days after sowing

DLI – Daily light integral ($mol \cdot m^{-2} \cdot d^{-1}$)

DPI – Daily photon integral ($mol \cdot m^{-2} \cdot d^{-1}$)

DW – Dry weight

EOD – End of day

FR – Far red

FW – Fresh weight

g – Gram

G – Green

GA – Gibberellin

HL – Hypocotyl length

HPS – High pressure sodium

HY5 – Long Hypocotyl 5

IAA – Indole-3-acetic acid

LED – Light-emitting diode

LMA – Leaf mass unit area

N – Nitrogen

PAR – Photosynthetically active radiation

PFD – Photon flux density

PIF – Phytochrome interacting factor

P_{FR} – Activated form phytochrome

P_R – Deactivated form phytochrome

PPFD – Photosynthetically photon flux density

PPS – Phytochrome photostationary state

SLA – Specific leaf area

R – Red

UV – Ultraviolet

LIST OF APPENDICES

CHAPTER ONE

INTRODUCTION

1.1 HISTORY OF MICROGREENS

Microgreens first appeared in commercial cuisine in the early 1980s, in San Francisco, USA (United States Department of Agriculture, 2014), with limited number of varieties. By the year 2000, they were still mainly available in upscale restaurants in the USA, as ingredients in soup, sandwiches and main dishes (Hansen, 2000; Yee, 2002). The market price dropped as the supplies of microgreens continued to rise, with an average of $30 to $50 USD per pound in Florida in 2016 (Treadwell et al., 2016). The market price may vary, but still remains relatively high due to the high production cost including investment of production system, labor cost and postharvest storage (Kaiser and Ernst, 2018).

As a new category of young and tender vegetable seedlings, microgreens are gaining substantial interests, because of their various colors, flavors and textures, and denser sources of nutrients compared to their mature counterparts (Treadwell et al., 2016; Xiao et al., 2012). They also have a short growth period, and are proposed to be a solution to the malnutrition issue facing the increasing global population (Ilakiya et al., 2020). Microgreens are harvested before or right after their first true leaves start to emerge, which normally takes 7–21 days depending on the species (Treadwell et al., 2016). There are now around 100 species that can be cultivated as microgreens, including vegetables, herbaceous plants, aromatic herbs and wild edible plants, belonging to the family of *Amaranthceae*, *Asteraceae*, *Apiaceae*, *Brassicaceae*, *Chenopodiaceae* and *Lamiaceae* (Kyriacou et al., 2016).

1.2 MICROGREEN PRODUCTION

Microgreens are normally grown in soilless culture systems, including using soilless substrates and solution culture (Di Gioia and Santamaria, 2015). Although microgreens can be grown in many different environments, the commercial production of high-quality microgreens is relatively difficult (Treadwell et al., 2016). Commercial microgreen production is usually conducted in controlled environments, like indoor vertical farms, greenhouses, and high tunnels, depending on the production size and climatic conditions (Di Gioia and Santamaria, 2015). Pest and disease management are very important since microgreens are cultivated in high density cropping system (Kaiser and Ernst, 2018). An adequate amount of light is required for all types of commercial cultivations of microgreens to ensure their normal growth, yield and nutritional quality. A study conducted by Xiao et al. (2012) on 25 microgreen varieties revealed that microgreens grown under light have higher nutritional content compared to the etiolated 'golden' varieties grown in darkness.

Commercial microgreen production has been increasingly switching from hand- to machine-harvesting to save time and reduce labour cost. Most microgreens are harvested at a height of 5–10 cm (Kyriacou et al., 2016). It is difficult to conduct machine harvest of microgreens if plant height is less than 5 cm (personal communication with some Canadian microgreen growers). Another major goal of commercial microgreen production is to optimize growth conditions that maximize fresh weight (FW) accumulation since microgreens are sold on a FW basis (Murphy and Pill, 2010). In addition, the color of the cotyledons could be another consideration, as red and darker green leafy greens are normally more appealing to consumers, as they are thought to contain higher nutrient levels compared to lighter colored green (Ali et

al., 2009). A recent study on the consumption of broccoli microgreens in the US reports that the sensory quality (i.e., smell, appearance, flavor and overall liking) and perceived benefits are two important factors that influence consumers' purchase intention (Chen et al., 2020).

1.3 PHYTOCHEMICAL PROFILES OF MICROGREENS

Vegetable crops are good sources of bioactive phytochemicals for human's daily diet. Many phytochemicals, including carotenoids, anthocyanins and ascorbate, are good sources of antioxidants that can promote human health by delaying or inhibiting oxidative damages and preventing chronic diseases (Alrifai et al., 2019; Hu, 2003). With increasing health issues, microgreens are gaining more and more attention and consumption, as they are known to have higher phytochemical contents compared to mature plants (Xiao et al., 2012). Over the past decade, crops in the *Brassicaceae* family have been extensively investigated and cultivated because of their phytochemical profiles (Björkman et al., 2011). The most commonly found phytochemicals in *Brassicaceae* crops include ascorbate, chlorophyll, carotenoid, total phenolics (e.g., flavonoid and anthocyanin). Environmental factors, including temperature, light, water availability and CO_2 concentration, greatly affect the biosynthesis of phytochemicals, and the responses also vary with species, developmental stage, plant density (Björkman et al., 2011).

1.4 LIGHT AND CONTROLLED ENVIRONMENT PLANT PRODUCTION

Light is one of the most important factors for plant growth, as it provides energy for photosynthesis and also acts as a signal that regulates morphological development (i.e., photomorphogenesis). Light intensity, spectrum and photoperiod are the three main factors

that could affect almost all aspects of plant growth and developmental processes (Singh et al., 2015). Artificial light sources, like high-pressure sodium (HPS), metal halide and fluorescent light, were traditionally used for controlled environment plant production as sole source or supplemental light sources (Kozai, 2013; Singh et al., 2015). The rapidly developing light-emitting diode (LED) lighting technologies have widely replaced traditional light sources in horticultural applications, because of their advantages of high energy efficiency, low heat output, long operating life time (Bourget, 2008). Moreover, the spectra of LED lights can be controlled to overlap with the typical part of the visible light spectrum that is absorbed by plant photoreceptors and pigments; in addition, the light intensities are adjustable, which can be applied in order to optimize plant growth and development based on the cultivars, growth stages and cultivation goals (Massa et al., 2008; Yeh and Chung, 2009). LED light has also become a valuable tool for the researchers to investigate plant morphological and physiological responses to varied light intensities and spectra (Massa et al., 2008).

LED lights that contain blue (B, 400–500 nm) light and red (R, 600–700 nm) light are the most commonly used spectral combinations for plant production in controlled environments. This is due in part to these wavelengths matching the maximum absorption of photosynthetic and accessory pigments, and most photoreceptors (Goins et al., 1997). Many studies have been conducted to investigate the optimal B and R ratio for plant growth and productivity, including in tomato (*Solanum lycopersicum* L.), cucumber (*Cucumis sativus* L.) seedling, lettuce (*Lactuca sativa* L.), spinach (*Spinacea oleracea* L.), kale (*Brassica oleracea* L.), basil (*Ocimum basilicum* L.), and bell pepper (*Capsicum annum* L.) (Graham et al., 2019; Hogewoning et al., 2010; Nanya et al., 2012; Naznin et al., 2019). There are also increasing amount of studies that incorporate

other wavelengths such as ultraviolet A and B (UV-A and UV-B, 280–400 nm), green (G, 500–600 nm) and far-red (FR, 700–800 nm) light, to the light recipe, and investigate the responses of varied species at different developmental stages (Snowden et al., 2016; Brazaityte et al., 2015; Li and Kubota, 2009).

1.5 LIGHT QUALITY AND PLANT PHOTOMORPHOGENESIS

There are several groups of photoreceptors in plants that absorb photons of certain wavelengths and use it as a signal to induce physiological responses. The known photoreceptors include UV-B photoreceptor protein (UVR8), phototropin, cryptochrome, phytochrome and Zeitlupe family (Möglich et al., 2010). Different groups of photoreceptors could work synergistically or antagonistically with each other to induce varied photo-responses, depending on the lighting environment (Fukuda et al., 2016; Casal and Mazzella, 1998).

Phytochromes are photoreceptors that strongly absorb R and FR light, and they interconvert between R light absorbing conformation (P_R, deactivated form) and FR light absorbing conformation (P_{FR}, activated form) (Smith and Holmes, 1977). The relative quantity of R and FR light that plants are exposed to, including the complete absence of specific wavelengths and temporal variations in light quality, controls the ratio of the P_R: P_{FR} forms of phytochromes. The ratio of P_{FR} to P_{total} (i.e., P_R and P_{FR}) in photosynthetic tissues is indicated by the phytochrome photostationary state (PPS) (Sager et al., 1988). Both forms of phytochromes absorb B light and ultraviolet-A (UV-A, 315–400 nm) light, although having much lower absorbance peaks than R and FR wavelengths (Fig 1.1). The activated P_{Fr} conformation move from cytoplasm into the nucleus to selectively interact with transcription factors (e.g., Phytochrome Interacting Factor; PIFs) and result in PIF inactivation, which is the initial step

underlying the photoreceptor's regulation of a wide range of phycological responses, including germination, stem elongation, leaf expansion and flowering (Legris et al., 2019; Wang and Deng, 2004). For example, ratios of R to FR light (R:FR) less than 1 are typically associated with understory and high-density cropping environments, which have been shown to elicit 'shade avoidance' responses in many genotypes; the most important of which is stem extension (i.e., increase in internode length) (Devlin et al., 1999). Low R:FR-mediated stem elongation associated with low phytochrome activity has been reported by many studies (Demotes-Mainard et al., 2016).

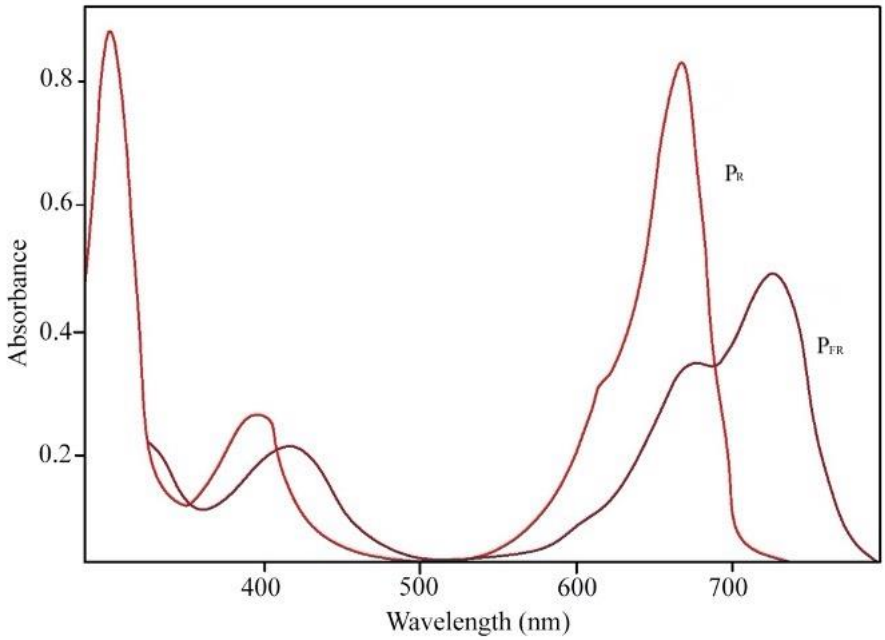


Figure 1.1. Absorption spectra of two forms of phytochromes: P_R (inactive form, light red) and P_{FR} (active form, in dark red). Adapted from Wang and Deng (2004).

Cryptochromes are B light photoreceptors that mediate several physiological responses. B light was commonly known to promote the formation of compact plants by suppressing hypocotyl elongation and cotyledon expansion during plant vegetative stage (Ahmad and

Cashmore, 1997; Shimizu et al., 2006). Interestingly, a growing body of evidence indicates that monochromatic B light promotes, rather than inhibits stem elongation in varied species (Fukuda et al., 2011; Hernández and Kubota, 2016; Johnson et al., 2020; Kong et al., 2018a; 2019a; 2019b). The effect of B light on promoting or inhibiting stem elongation may be associated with the phytochrome activity, which is affected by the addition of other light wavelengths (e.g., R light) (Kong et al., 2019a; 2019c; Graham et al., 2019). Moreover, B light also regulates other morphological responses, like phototropism and flowering initiation, which may also involve other photoreceptors including phototropins and phytochromes (Guo et al., 1998; Kong and Zheng, 2020).

1.6 LIGHT QUALITY AND PLANT GROWTH AND YIELD

Yield is an important parameter for plant production, especially fruits and vegetables, since the majority of these are sold on a FW basis. R LED light was first available for practical use in 1962, and it became widely used for plant production following the development of super bright LEDs in the 1980s (Yeh and Chung, 2009), although the efficiency of R LED light was low. However, plants under 100% R LED display a “R light syndrome” like dysfunctional photosynthetic operations, undesirable growth characteristics, and the translocation of photosynthates out of the leaves might also be inhibited (Hogewoning et al., 2010; Sæbø et al., 1995; Trouwborst et al., 2016).

With the later development of B LED light, adding B to R LED was found to prevent “R light syndrome” and relieve some undesired growth characteristics under pure R including down-rolled leaf margins, low photosynthetic rate and low biomass (Brown et al., 1995; Chang et al., 2016; Hogewoning et al., 2010). The combination of R and B LED light (RB-LED) increased

the dry weight (DW) of spinach, lettuce and radish as compared to plants cultivated under R LED light alone under the same photosynthetic photon flux density (*PPFD*) (Yorio et al., 2001). Moreover, B light is required in optimizing photosynthesis, mediating stomatal opening, chloroplast movement and nutrient uptake (Davis and Burns, 2016). RB-LED lights are predominantly employed in commercial plant growth. RB-LED light also increased both FW and DW of chili pepper (*C. annum*), lettuce and hybrid moth orchid (*Phaelenopsis × Doritis*), and FW of sprouting broccoli (*Brassica oleacea* L.) (Gangadhar et al., 2012; Johkan et al., 2010; Kopsell et al., 2014; Lin et al., 2013; Shin et al., 2008) relative to traditionally used fluorescent light. However, there are a lack of studies that investigate the optimal R and B regimes (i.e., R to B ratio) on the growth and yield of commercially grown microgreen species.

G light can penetrate chloroplasts located deeper within the leaf mesophyll as compared to R and B light, and potentially enhances the leaf photosynthetic rate (Terashima et al., 2009). FR light is also used by plants as a key stimulus for inducing changes in light acclimation and is associated with net photosynthesis when it combines with R and B light (Murakami et al., 2016; Zhen and van Iersel, 2017). Therefore, adding G or FR light to RB-LED can also improve plant growth and yield. For example, the shoot FW of red leaf lettuce was increased by 61% under $B_{10}G_{10}R_{80}$ compared to $B_{20}R_{80}$ under a *PPFD* of around 173 $\mu mol \cdot m^{-2} \cdot s^{-1}$ (Son and Oh, 2015). Also, adding 12 to 149 $\mu mol \cdot m^{-2} \cdot s^{-1}$ FR to BR-LED ($B_{20}R_{80}$) at a *PPFD* of 130 $\mu mol \cdot m^{-2} \cdot s^{-1}$ increased the aboveground FW and DW of lettuce accordingly (Lee et al., 2016). However, adding G or FR light did not significantly affect the FW of microgreens (Ying et al., 2020a), or increased the FW limited to specific species under certain *PPFD* (Gerovac et al., 2016).

1.7 LIGHT QUALITY AND SECONDARY METABOLITE AND NITRATE ACCUMULATION

Plant secondary metabolites do not participate directly in cell growth or division, but are produced to protect against biotic and abiotic stresses, and play a significant role in delaying or inhibiting oxidative damage. The enhancement of secondary metabolites for daily diets of humans could modulate inflammation in the immune system for the human body, when facing oxidative stresses (Zhang and Tsao, 2016). The biosynthesis of secondary metabolite is mainly controlled by transcriptional factors, which are regulated by internal (e.g., plant hormones) and external signals (e.g. light) (Vom Endt et al., 2002).

Chlorophyll and carotenoid are important pigments in plant photosynthesis. The biosynthesis of chlorophyll initiates from glutamate, and involves in other chlorophyll precursors like 5-aminolevulinic acid (ALA) and protochlorophyllide (Tanaka et al., 2011). Chlorophyll molecules absorb light across the photosynthetically active radiation spectrum (*PAR*, 400–700 nm), although certain wavelengths (B and R) are more strongly absorbed as compared to others. The absorption peaks of chlorophyll *a* and chlorophyll *b* are 453 nm, 642 nm, and 430 nm, 662 nm, respectively (Comar and Zscheile, 1942). Chlorophyll *a* and *b* play a predominant role in photosynthetic light absorption. The biosynthesis of chlorophyll requires light, and transcriptional factors that mediate light-induced responses, are involved in chlorophyll biosynthesis (Liu et al., 2017).

Carotenoids are a specialized group of tetraterpenes that are derived from the mevalonate pathway. They act as accessory light-harvesting pigments that effectively absorb the photons ranging from approximately 350–500 nm, pass the energy to chlorophylls and drive

photosynthesis (Griffiths et al., 1955). They also play an important role in mitigating oxidative damages through the xanthophyll cycle when plants are exposed excessive light (Cogdell and Gardiner, 1993). It is relatively well understood that phytochrome-mediated transcription factors PIF and Long Hypocotyl 5 (HY5) are involved in carotenoid biosynthesis (Stanley and Yuan, 2019). Therefore, light intensity and quality are important factors that impact carotenoid content in plants (Brazaitytė et al., 2015).

Phenolic compounds comprise a diverse group of secondary metabolites in plants that have a large range of structures and functions. They consist of water-soluble compounds (e.g., flavonoids), phenolic acid and their derived water-insoluble compounds (e.g., lignins), and they act as antioxidant and free radical scavengers in the plant (Cheynier et al., 2013; Rispail et al., 2005). Specifically, flavonoids absorb shorter wavelength of light including UV light, although the energy cannot be used for photosynthesis (Agati et al., 2012). Flavonols, a subclass of flavonoids, are ubiquitous in vegetables and the flavonol quercetin is the most common and biologically active flavonol in the human diet (Ho, 1992). Anthocyanins, another subclass of flavonoids, are commonly associated with the red or purple color formation in leaves and flowers. Phenolic compounds are synthesized through the phenylpropanoid pathway, where the production of flavonoids, including anthocyanins is regulated by gene transcription (Cheynier et al., 2013). The accumulation of phenolic compounds, like other secondary metabolites, are greatly affected by environmental alternations, including light intensity and spectra (Zhao et al., 2005).

Ascorbate, which is commonly known as vitamin C, is an essential nutrient with strong antioxidant activity for human health. However, humans acquire ascorbate via dietary uptake

because they are not able to synthesize it (Nishikimi et al., 1994). Plants produce ascorbate in order to limit oxidative stresses, including those associated with biotic and abiotic challenges (Giovannoni, 2007). Ascorbate is produced from glucose through intermediate precursors including L-galactono-1,4-lactone and L-galactono-1,4-lactone dehydrogenase, though the full pathway has not yet been established (Yabuta et al., 2007). Nevertheless, the biosynthesis of ascorbate has been proven to be modulated by light at the transcriptional level (Wang et al., 2013).

Nitrogen (N) is the most important macronutrient in plant nutrition and nitrate-N is the main source of N uptake in plants. N is involved in the synthesis of proteins, enzymes and chlorophyll in plants (Yuan et al., 2012). However, opposite to the aforementioned secondary metabolites, the accumulation of nitrate through leafy vegetables could be harmful to human health. Nitrate itself is generally regarded non-toxic, but if a proportion (i.e., around 5%) of nitrate is reduced to toxic nitrite anion (NO_2^-), it raises concern for increased risk of gastrointestinal cancers (Mensinga et al., 2003; Pinto et al., 2015). A previous study shows the impact of light quality on nitrogen metabolism, and the effects are dependent on the developmental stages of the leaves (Maevskaya and Bukhov, 2005).

It has been experimentally found that regulating light quality has a pronounced effect on cellular metabolism and biosynthesis of secondary metabolites using LED light (Alrifai et al., 2019). For example, increased R LED light or supplemented R LED light with HPS light increased ascorbic acid and total phenolic content in basil (*Ocimum basilicum*) and parsley (*Petroselinum crispum*) microgreens compared to HPS light (Samuolienė et al., 2016). Total ascorbate content in lettuce increased when B light percentage increased from 25% to 50% and 75% under a R

and B LED light regimen for 12 d before harvest (Zha et al., 2020). Chlorophyll content of spinach leaves increased when a proportion of B light (10% to 50%) was added to the dominant R light background (Matsuda et al., 2007). Under the same *PPFD*, a mixture of R and B light increased chlorophyll content in leaf lettuce, decreased in komatsuna (*Brassica campestris* L. cv. Komatsuna) and did not affect spinach (*Brassica campestris*) as compared to grown under white fluorescent light. The species-specific response to light quality was also established for levels of carotenoids, ascorbic acid and nitrate in the same study (Ohashi-Kaneko et al., 2007). An increase in the concentration of phenolic compounds of lettuce occurred under varied light spectral environments, including increasing B light percentage in RB-LED lighting (Son and Oh, 2013) and under fluorescent light supplemented with R light as compared to with UV and far-red light (Li and Kubota, 2009). B light increased total flavonoid content in Cyclocarya (*Cyclocarya paliurus* (Batalin) Iljinsk.) leaves compared to white and R LED under the same *PPFD* at 40 d after treatment, while showing no difference at 20 d after treatment (Liu et al., 2018). Overall, it seems that the effects of light quality on plant secondary metabolites vary greatly among growth stages, species and even cultivars. Therefore, the effect of light quality on phytochemical contents in microgreens, and the underlying mechanisms of such specific effects of light spectra modulating secondary metabolites, need further study.

1.8 OVERVIEW OF THE RESEARCH

1.8.1 Hypothesis and objectives

The overall hypothesis of the thesis was: the growth, yield, appearance quality and phytochemical contents of microgreens can be promoted by adjusting the light spectral quality from LED light (sole-source and supplemental; daytime and nighttime), but the responses could

be species-specific. A series of experiments were designed to investigate the effect of light spectral quality during daytime and nighttime on growth and appearance quality, yield, phytochemical and nitrate contents of commercially grown microgreen species.

The specific objectives were to:

1. Investigate the effect of spectral quality of sole-source RB-LED lights, from 5% B and 95% R (B_5R_{95}) to $B_{30}R_{70}$ on growth, appearance quality and yield of four different species of *Brassicaceae* microgreens under growth chamber condition.
2. Investigate the effect of spectral quality of sole-source RB-LED lights, B_5R_{95} to $B_{30}R_{70}$ on phytochemical (i.e., total carotenoids, chlorophyll *a* and *b,* ascorbate metabolites, total phenolics, anthocyanins) and nitrate contents of four different species of *Brassicaceae* microgreens under growth chamber condition.
3. Explore the light regimes (quality, duration and initiation time) of supplemental light during nighttime that promote microgreen elongation, without compromising their yield and quality under growth chamber condition.
4. Evaluate the effect of low intensity supplemental B /FR light overnight in the greenhouse on stem elongation, appearance quality, yield and phytochemical contents (i.e., total carotenoids, phenolic, chlorophyll *a* and *b*) of microgreens during winter months under greenhouse condition.
5. Investigate the effect of monochromatic B light on stem elongation and its underlying mechanism through the regulation of phytohormones, phytochemical (i.e., flavonoids), and their interaction.

1.8.2 Significance

Controlled environment plant production provides the opportunity for year-round, sustainable crop cultivation, while addressing increasing concerns of growing populations and environmental degradation. This study was designed to improve the growth, appearance quality and phytochemical contents of microgreens by manipulating spectral quality of LED light in controlled environments. The light quality may not be identical for all species, or the same species at different growth stages and production goals. For example, the optimal light recipe that works for one species may not work for another species or cultivars under the same environment. Similarly, an optimized light quality to maximize yield may not lead to the highest nutritional profiles. Therefore, the results of the present study would provide a profound understanding on species-dependent responses under varied light quality during the seedling stage of plants. Improved knowledge of microgreen responses to light can thus be a powerful tool for commercial growers to improve microgreen growth and development and be more cost effective. It also can lead to more exciting new opportunities for both research and commercial production.

Note: Each chapter in this thesis is an independent manuscript which has either been published (Chapters 2 in Scientia Horticulturae, chapter 4 and 5 in Hortscience) or submitted to peer-reviewed journal and currently under review (Chapters 3). A detailed experimental protocol for future research is also included as an appendix.

CHAPTER TWO

RESPONSES OF YIELD AND APPEARANCE QUALITY OF FOUR BRASSICACEAE MICROGREENS TO VARIED BLUE LIGHT PROPORTION IN RED AND BLUE LIGHT-EMITTING DIODES LIGHTING[1]

ABSTRACT

To optimize B light proportion in RB-LED lighting for microgreen production, the yield and appearance quality of cabbage, kale, arugula and mustard were investigated under RB-LED lightings with 5%, 10%, 15%, 20%, 25% and 30% B light. For each lighting treatment, the PPFD was set at ≈ 300 $\mu mol \cdot m^{-2} \cdot s^{-1}$, and the air temperature during light/dark was set at ≈ 21/ 17 °C. Neither fresh nor dry yield was generally affected by B light percentage for the tested species except cabbage, which showed quadratic (peaking at 15%) responses in crop yield. Hypocotyl length and cotyledon area of kale and mustard decreased linearly with increasing B light percentage which, however, did not affect arugula and cabbage in these two traits. For plant color, cotyledons were darker red for mustard and less pure green for the other three species under higher B light percentage. This was indicated by a negative linear response of hue angle or green chromaticity to increasing B light percentage. These findings suggested that responses to B light percentage varied with plant traits and microgreen species. To reach a balance on the consideration of yield and appearance quality, 15% B light was recommended for indoor production of cabbage microgreens, while 5% B light for the other three species, under similar environmental conditions.

---

[1] *Article citation*: Ying, Q., Y. Kong, C. Jones-Baumgardt, and Y. Zheng. 2020. Responses of yield and appearance quality of four Brassicaceae microgreens to varied blue light proportion in red and blue light-emitting diodes lighting. Sci. Hort. 259:1168-1174. https://doi.org/10.1016/j.scienta.2019.108857.

2.1 INTRODUCTION

Microgreen consumption has increased in recent years because of their various colors, attractive flavors and unique textures, as well as nutritional benefits to human health (Treadwell et al., 2016; Xiao et al., 2012). Although 80 to 100 plant species can be cultivated as microgreens, the most popular species are from the *Brassicaceae* family such as cabbage, kale, arugula and mustard. For indoor plant production (e.g., microgreens), artificial lighting has been increasingly used in order to improve crop growth and quality, without being restricted by the ambient light condition.

LED lights have been developing into an alternative to traditional artificial lights (e.g., HPS light) in crop production because of their advantages such as space saving, safety, longevity, low radiant heat output (Barta et al., 1992), as well as adjustable light quality which could manipulate plant morphology and productivity (Tarakanov et al., 2012). R LED light was first available for early researchers to investigate its effects on plants physiology (Olle and Viršile, 2013; Folta and Childers, 2008). Under R LED as a sole light source, plants exhibited some undesirable growth characteristics such as lower biomass, lower net photosynthetic rate, or downward leaf curling, but these symptoms were relieved when B light was added (Brown et al., 1995; Chang et al., 2016; Goins et al., 1997; Hoenecke et al., 1992; Hogewoning et al., 2010; Kim et al., 2004; Kong et al., 2018a; Wang et al., 2015). Also, B and R wavelengths can be readily absorbed and utilized by plant leaves (McCree, 1971). With the later development of super bright B LED, the combination of R and BLED (RB-LED) lighting has been increasingly used for horticultural crops production, including microgreens (Gerovac et al., 2016; Kong et al., 2018b; Massa et al., 2008; Samuolienė et al., 2013; Tanaka et al., 1998).

In previous studies on other crop production, plant biomass responses to B light percentage in RB-LED lighting were not consistent. There were no differences in FW or DW for rapeseed plants under 25%, 50% and 75% B light (Li et al., 2013) and in FW for sprouting broccoli between 5% and 20% B light (Kopsell et al., 2014), when using RB-LED lighting at a *PPFD* of 60 and 100 $\mu mol \cdot m^{-2} \cdot s^{-1}$, respectively. However, a decrease was found in FW and DW for "Sunmang" lettuce with increasing B light from 13% to 59% at a *PPFD* of 171 $\mu mol \cdot m^{-2} \cdot s^{-1}$ (Son and Oh, 2013) and a similar response in FW for cucumber seedlings with B light increased from 10% to 75% at a *PPFD* of 100 $\mu mol \cdot m^{-2} \cdot s^{-1}$ (Hernández and Kubota, 2016) when using RB-LED lighting. It appears that the biomass responses to B light percentage vary with plant species and light intensity. Microgreens are specialty crops with short growth period, so the effect of light spectra on their yields might result more from photomorphogenesis than from photosynthesis. Thus, the responses of microgreens to B light percentage may differ from mature plants, which has not been fully understood and requires further studies.

Previous studies reported that morphological response to different B light percentages also varied with species. When B light percentage increased from 10% to 30% and 50% under RB-LED lighting, the hypocotyl lengthening was inhibited in tomato seedlings (Nanya et al., 2012). Similarly, for cucumber seedlings, hypocotyl length (HL) and leaf area decreased linearly as B light percentage increased from 10% to 75% under RB-LED lighting (Hernández and Kubota, 2016). Cope and Bugbee (2013) also reported decreased HL and leaf area of radish and soybean under increasing B light percentage of white LED light, while wheat was not affected. To our best knowledge, the effect of varying B light percentages in RB-LED lighting on leaf color, which is one of the important appearance traits for microgreens, has so far been unknown.

Despite many previous studies on other crop production systems, limited information is available about RB-LED lighting effects on microgreens. Our recent research showed that the optimal light intensity for cabbage, kale, arugula and mustard microgreens grown in a walk-in growth chamber based on aboveground fresh and dry biomass was 300 $\mu mol \cdot m^{-2} \cdot s^{-1}$ with a 16 h photoperiod (Jones-Baumgardt et al., 2019). Crop yield and appearance quality are generally the two most important factors microgreen growers care about. Surprisingly, based on these two factors, the optimal B light percentage in RB-LED lighting has so far been unclear for indoor production of microgreens.

It has been found that plant growth and yield decreased when increased B light percentage reached a certain level (e.g., 35%) (Dou et al., 2017). For instance, increasing B light from 13% to 35% resulted in only one third of the FW biomass and half of the leaf area of lettuce (Son and Oh, 2013). The FW of sprouting broccoli decreased by 20% when increasing B light proportion from 5% to 20% (Kopsell et al., 2014). In these cases, electric-energy-to-plant-biomass conversion efficiency would decrease with increasing B light percentage, because B vs. R LED had a lower or similar electric-energy- photosynthetically active radiation (PAR) conversion efficacy ($\mu mol \cdot J^{-1}$) (Hernández and Kubota, 2014). Also, sunlight contains around 31% B light out of *PAR* (ASTM, 2003). It appears that around 30% may be the maximum B light percentage to produce plant biomass efficiently and keep appearance quality in an acceptable range.

Based on all the above information, when RB-LED lighting, with a *PPFD* of 300 $\mu mol \cdot m^{-2} \cdot s^{-1}$ and photoperiod of 16 h, was used for indoor production of arugula, cabbage, kale and mustard microgreens, three hypotheses were proposed as follows: (1) crop yield responses to B light

percentage differ among species; (2) appearance quality responses to B light percentage vary with species; (3) when B light percentage varies from 5% to 30%, there is an optimal B light level for each species based on its different responses in crop yield and appearance quality. The objective of the present study was to elucidate the optimal B light proportion in RB-LED lighting for indoor microgreen production by testing the above hypotheses.

2.2 MATERIALS AND METHODS

2.2.1 Plant material and growing conditions

The experiment with three replicates over time was conducted on the four microgreens species (Table 1) at the University of Guelph, Guelph, Ontario, Canada during the period from Sep. 2017 to Jan. of 2018. Four pre-seeded fiber trays (48.5 × 23 × 3.5 cm) with the above four microgreens species (one species in each tray), from Greenbelt Microgreens Inc. (Lynden, ON, Canada) were placed inside a walk-in growth chamber (728 × 399 × 252 cm) to start the lighting treatments with a photoperiod of 16 h. The substrate consisted of 30% compost, 30% peat, 30% coir and 10% perlite. Organic fertilizer had been pre-incorporated to the substrates, which had a pH = 6.9, and electrical conductivity (EC) = 1.9 $dS \cdot m^{-1}$, and each nutrient content ($mg \cdot kg^{-1}$) in the substrates was as follows: N 9400, P 329, K 1410, S 220, Mg 295, Ca 1390, Zn 9.4, Mn 10, Fe 47, Cu 0.6, Na 218, and B 0.7. All plants were top irrigated at least once each day using well water (pH = 7.5; EC = 0.8 $dS \cdot m^{-1}$) until visible drainage. The temperature was set at 21 °C during light period (10 a.m. to 2 a.m.) and 17 °C during dark period, while the relative humidity (RH) was set at 80% during both light and dark period. The 30% increase of vapor pressure deficit from dark to light period had little impact on transpiration during dark period since the stomata were generally closed, while the most impacts on transpiration during light period were well

controlled. The temperature and RH were controlled by the Argus control system (Argus Controls Systems Ltd., Surrey, BC, Canada). Temperature / RH/ 2 external channels data loggers (Onset HOBO U12-013, Onset Computer Corporation, MA, USA) were also used in the growth chamber to record actual temperature and RH every 5 minutes.

Table 2.1. Information of the tested four microgreen plant species and the key time point for the experiment.

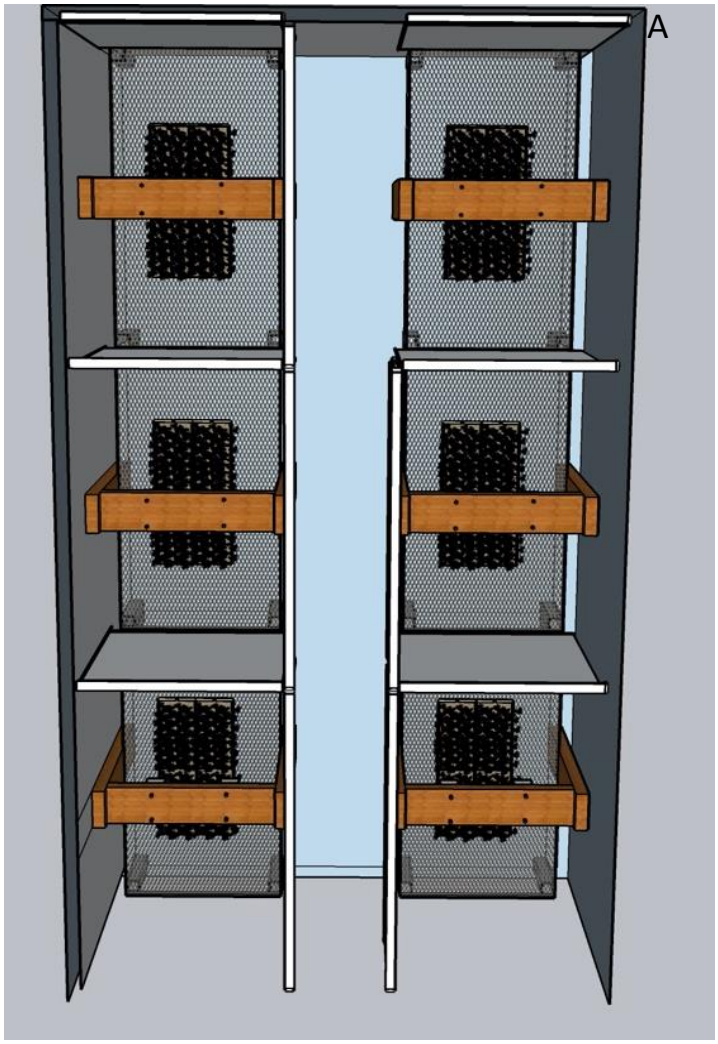
Plant materials	Scientific name	Plant color	Seed source	Seeding date	Harvesting date
Cabbage	*Brassica oleracea* var. *Capitata* f. *Rubra*	Purple stem and leaf margin with green leaf	Mumm's Sprouting Seeds, Parkside, SK, Canada	Rep.1: Oct. 2[a] Rep.2: Nov. 30 Rep.3: Jan. 9	Rep.1: Oct. 12 Rep.2: Dec. 10 Rep.3: Jan. 19
Kale 'Red Russian'	*Brassica napus*	Purple stem with green leaf	High Mowing Organic Seeds, Wolcott, VT, USA	Rep.1: Oct. 2 Rep.2: Nov. 30 Rep.3: Jan. 9	Rep.1: Oct. 12 Rep.2: Dec. 10 Rep.3: Jan. 19
Arugula	*Eruca sativa*	Green leaf	Suba Seeds Company S.P.A., Longiano, FC, Italy	Rep.1[z]: Oct. 2 Rep.2: Nov. 30 Rep.3: Jan. 9	Rep.1: Oct. 13 Rep.2: Dec. 11 Rep.3: Jan. 20
Mustard 'Mizuna'	*Brassica juncea,*	Purple leaf vein or whole leaf	Johnny's Selected Seeds, Winslow, ME, USA	Rep.1: Oct. 2 Rep.2: Nov. 30 Rep.3: Jan. 9	Rep.1: Oct. 13 Rep.2: Dec. 11 Rep.3: Jan. 20

[a] Experimental replicate.

2.2.2 Experimental design and treatments

The experiment was conducted as a completely randomized block design with one factor (light quality) and three replicates over time. Light quality treatments included: (1) 5%; (2) 10%; (3) 15%; (4) 20%; (5) 25%; (6) 30% B light in RB-LED lighting (Heliospectra LX602C, Heliospectra

AB, Gothenburg, Sweden). For each replicate, the six light quality treatments were randomly allocated to six compartments separated by an opaque curtain to prevent neighbouring effects (Fig 2.1). The light quality treatments were replicated by switching light spectra output of LED arrays in each compartment within the chamber (i.e. changing the position of light quality treatments within six compartments) during the three replications of the experiment.



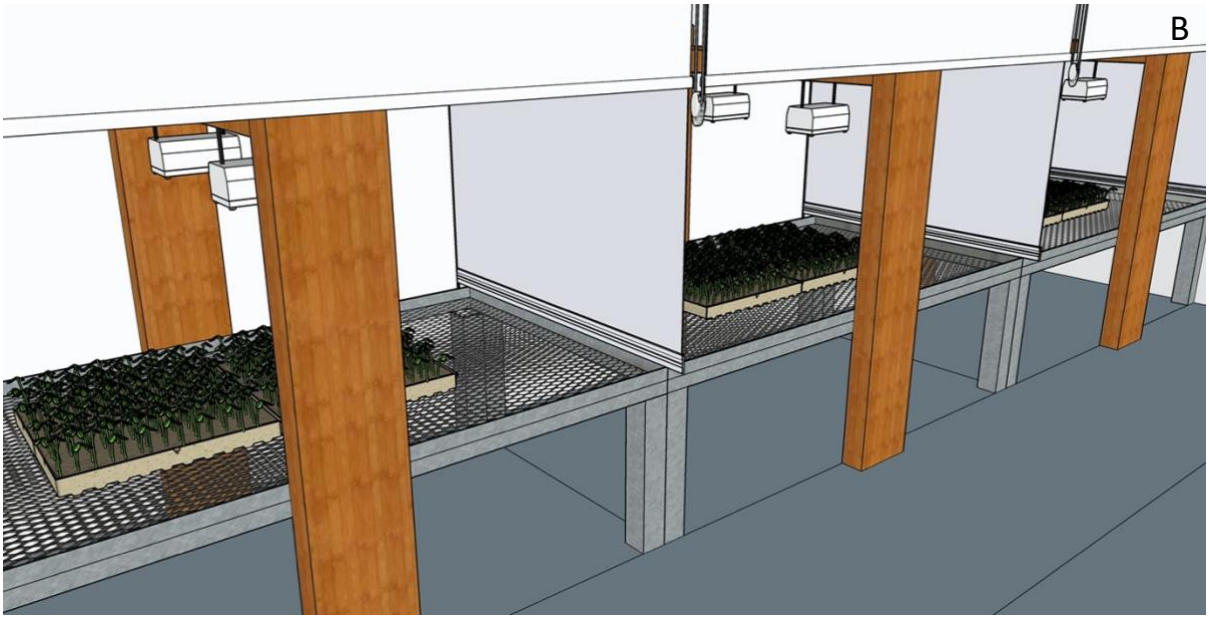


Figure 2.1. Top view (A) and side view (B) of the schematic of experimental growth chamber.

In the treatment area, the lights were placed 56.5 cm above the top of the substrate (measured from the bottom of the LED array) to achieve an average target *PPFD* of around 300 $\mu mol \cdot m^{-2} \cdot s^{-1}$, which provided a total daily light integral (DLI) of around 17.3 $mol \cdot m^{-2} \cdot d^{-1}$. The light intensity and spectrum of the LED arrays were set up by Heliospectra System Assistant (Version 1.3.0.). To check light uniformity, light intensity and spectrum were measured at 25 spots within an area of 0.65 m^2 under each treatment for each replicate using a Flame-S spectrometer with a 25 µm slit, coupled to a 1.89 m × 400 µm solarization resistant fiber optic patch chord with a CC-3 Cosine Corrector with spectralon diffuser (Ocean Optics, Inc., Dunedin, USA). The spectrometer was calibrated over 350 to 800 nm using a HL-3-VIS calibrating light source (Ocean Optics, Inc.). Light intensity and spectrum were checked at the beginning and the end of each replicate. The light intensity, spectral distribution, air temperature and RH under different light treatments are presented in Table 2.2.

Table 2.2. Mean photosynthetic photon flux density (*PPFD*) of combined (400–700nm), B light (400–500nm) and B light proportion, temperature and relative humidity (RH) of different light quality treatments.

Treatment	*PPFD*[a] ($\mu mol \cdot m^{-2} \cdot s^{-1}$)	B light intensity ($\mu mol \cdot m^{-2} \cdot s^{-1}$)	B light proportion (%)	Air temp. during light (°C)	Air temp. during dark (°C)	RH (%)
5% B	301.7 ± 2.2	15.7 ± 0.3	5.2 ± 0.1	20.2 ± 0.3	16.7 ± 0.4	76.8 ± 5.6
10% B	298.7 ± 0.9	29.8 ± 0.2	10.0 ± 0.1	20.2 ± 0.0	16.8 ± 0.2	81.9 ± 4.3
15% B	298.9 ± 1.2	45.5 ± 0.4	15.2 ± 0.0	20.4 ± 0.4	16.7 ± 0.4	82.2 ± 3.5
20% B	300.3 ± 0.7	60.2 ± 0.3	20.1 ± 0.0	20.9 ± 0.3	16.9 ± 0.2	76.6 ± 6.5
25% B	300.9 ± 2.6	75.2 ± 1.0	25.1 ± 0.1	20.7 ± 0.4	16.6 ± 0.3	84.2 ± 1.3
30% B	301.0 ± 2.0	91.6 ± 1.1	30.4 ± 0.2	20.1 ± 0.4	16.4 ± 0.5	77.1 ± 3.0

[a] *PPFD* of combined and B light reported are the mean and standard error of 25 scans under treatment area

[b] Error bars indicate ± standard error (n = 3).

2.2.3 Parameter Measurements

Microgreens were harvested 10 d after seeding for cabbage and kale, and 11 d after seeding for mustard and arugula (Table 1). Plants were harvested at the same time on each day for each replicate. For each replicate and each species, three cores (76.5 cm^2 surface area for each core) of microgreens together with substrates were randomly sampled from each tray (treatment). All the microgreens within each core were cut right above the substrate level and collected to determine FW. To determine DW, these plants were then put in paper bags and dried at 65 °C until a constant weight was achieved. The fresh yield (kg m^{-2}) and dry yield (g m^{-2}) were calculated based on average FW and DW of microgreens harvested from sampled cores and core areas. Five plants from each species were randomly selected from the remainder of

microgreens in each tray for each replicate to measure the HL using a ruler. Then their cotyledons were cut off from stem to measure the total leaf area using a leaf area meter (Li-Cor 3100C; Li-Cor Inc, Lincoln, NE, USA), and the average leaf area of individual plants was calculated and recorded. From the remainder of microgreens in each tray for each replicate, another five plants from each species were selected randomly to analyse the cotyledon color (one leaf from each plant). After cutting off the stem, the leaves were scanned to capture color images using a scanner (Canoscan F910111, Canon Inc., Japan) with 300 dpi resolution. The digital images were saved as JPG format in computer. The R, G, B values (i.e., the amount of red, green, blue light emitted for each pixel in the image which is measured on a scale of 0 to 255) were obtained from the digital image of each cotyledon using ImageJ 1.42 software (National Institute of Health, USA). Subsequently, hue angle was calculated from these R, G, B values using the formulas according to Karcher and Richardson (2003). The cotyledon hue angle, the attribute of color perception refers to a position on a continuous circular scale, is an accurate indicator of cotyledon visual color (Landschoot and Mancino, 2000), was calibrated with the actual hue angle of the Munsell color chips using Munsell Conversion software for 2018 (Munsell color, 2018). The leaf chromaticity is another important parameter to differ leaf color (Lee and Lee, 2013; Sá Junior et al., 2011), particularly, green color (Green%) for green-leafed microgreens, including arugula, cabbage and kale, was calculated as eqs. (1).

$$\text{Green\%} = G/(R + G + B) \times 100 \qquad (1)$$

The leaf chromaticity of red color (Red%) for red-leafed mustard, was calculated as eqs. (2):

$$\text{Red\%} = R/(R + G + B) \times 100 \qquad (2)$$

2.2.4 Statistical Analyses

Data were subjected to analysis of variance using the SPSS software (Version 25.0, IBM, New York, USA) and were presented as mean ± SE (standard error). When there were significant treatment effects, regression analysis was used to determine the relationship between plant traits and B light percentage, and regression lines were presented in figures by GraphPad Prism statistical software (GraphPad Software, San Diego, CA, USA) together with regression equations and determination coefficients (R^2).

2.3 RESULTS

2.3.1 Crop yield

In the present study, B light percentage did not affect the fresh yield (kg m^{-2}) for the tested microgreen species except for cabbage (Fig. 2.2). For cabbage, its fresh yield showed a nonlinear response to B light percentage, with a peak at 15% B light. Cabbage microgreens grown under 15% B light had greater FW (by 18.6% and 11.9%) than those under 5% and 30% B light, respectively.

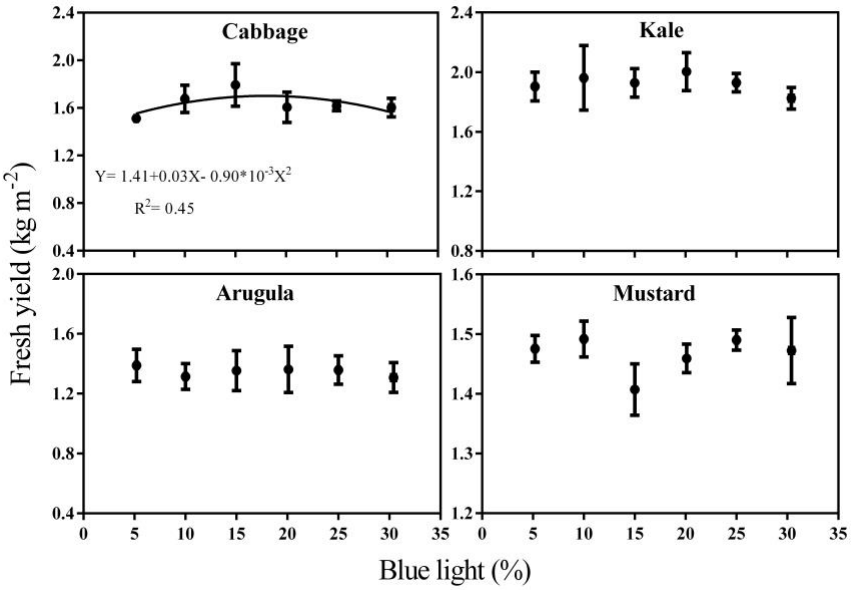


Figure 2.2. The response of fresh yield to red and blue (B) LED lighting with varying B light percentage in four microgreens species. The total photosynthetic photon flux density (*PPFD*) of each lighting treatment was ≈ 300 µmol·m^{-2}·s^{-1}. Data are means ± SE of three separate experimental replicates. Regression lines are shown where the effects of B light percentage are significant at $P \leq 0.05$ and there are significant linear or non-linear regressions. The determination coefficient for the regression is shown as R^2.

For cabbage, the dry yield (g m^{-2}) followed a similar pattern of the response to B light percentage as fresh yield, with a peak at 15% B light (Fig. 2.3). The dry yield of cabbage under 15% B light was 9.6% and 7.0% greater than that under 5% and 30% B light, respectively. There was no significant difference in dry yield among different light treatments for the other three species (Fig. 2.3).

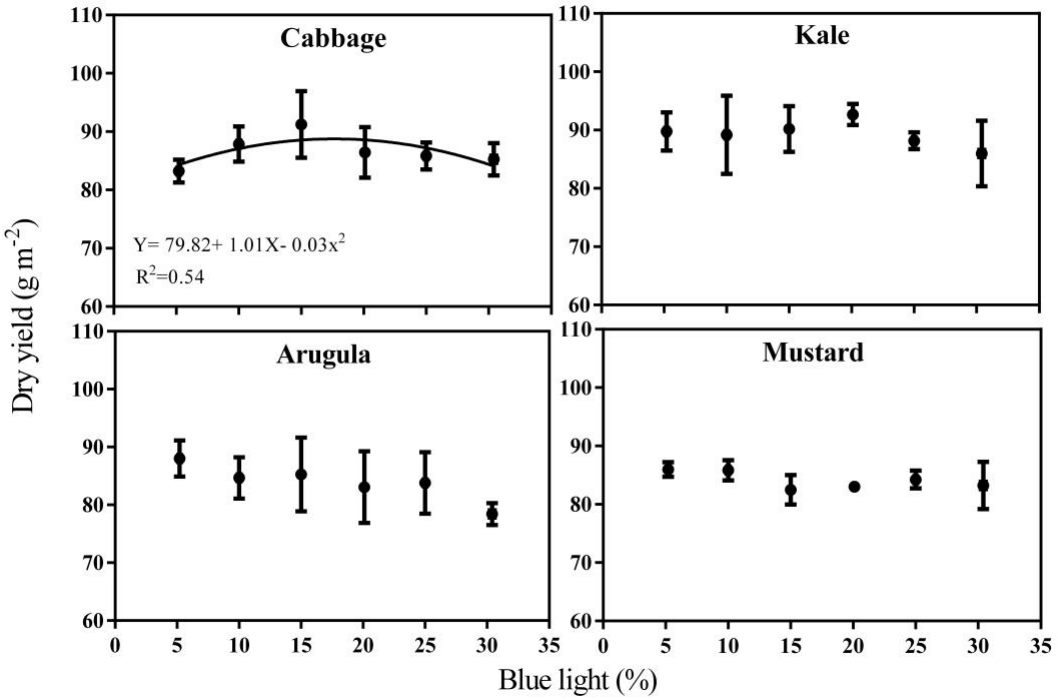


Figure 2.3. The response of dry yield to red and blue (B) LED lighting with varying B light percentage in four microgreens species. The total photosynthetic photon flux density (*PPFD*) of each lighting treatment was ≈ 300 µmol·m^{-2}·s^{-1}. Data are means ± SE of three separate experimental replicates. Regression lines are shown where the effects of B light percentage are significant at $P \leq 0.05$ and there are significant linear or non-linear regressions. The determination coefficient for the regression is shown as R^2.

2.3.2 Plant size

For kale and mustard, the HL decreased linearly with increasing B light from 5% to 30% (Fig. 2.4). Plants grown under 5% B light had 7.6% and 16.3% longer HL for kale and mustard, respectively, compared to 30% B light. However, this response of HL to B light percentage was not found in cabbage and arugula (Fig. 2.4).

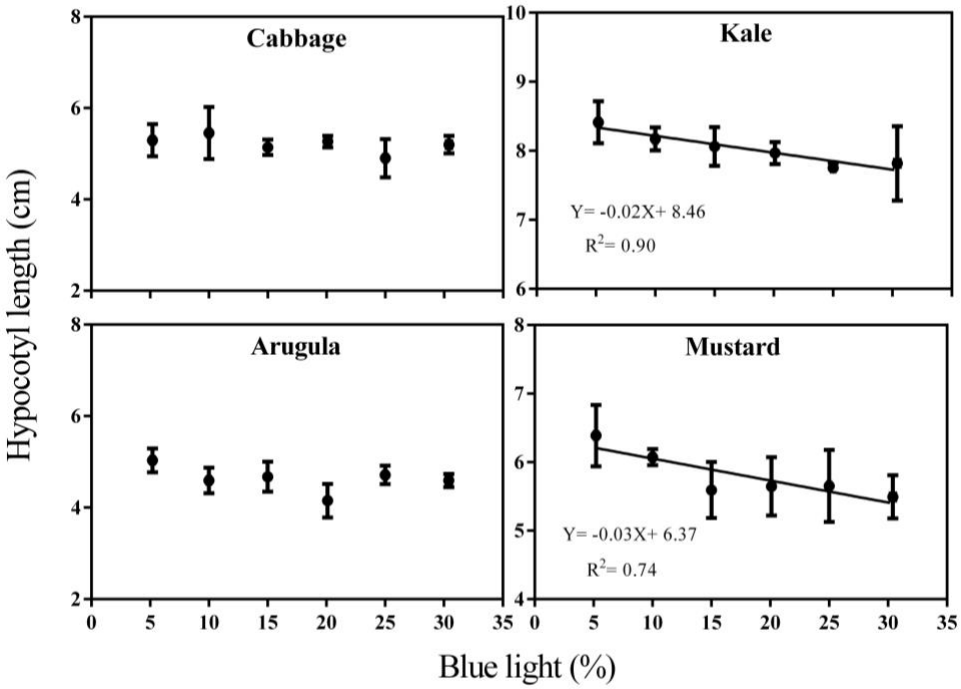


Figure 2.4. The response of hypocotyl length to red and blue (B) LED lighting with varying B light percentage in four microgreens species. The total photosynthetic photon flux density (*PPFD*) of each lighting treatment was ≈ 300 µmol·m^{-2}·s^{-1}. Data are means ± SE of three separate experimental replicates. Regression lines are shown where the effects of B light percentage are significant at $P \leq 0.05$ and there are significant linear or non-linear regressions. The determination coefficient for the regression is shown as R^2.

The response of cotyledon area to increased B light percentage differed among different species (Fig. 2.5). For kale and mustard, the cotyledon area decreased linearly as B light percentage increased, and was 16.3% and 19.3% smaller under 30% vs. 5% B light treatment, respectively. However, this response in cotyledon area to B light percentage was not found in cabbage and arugula.

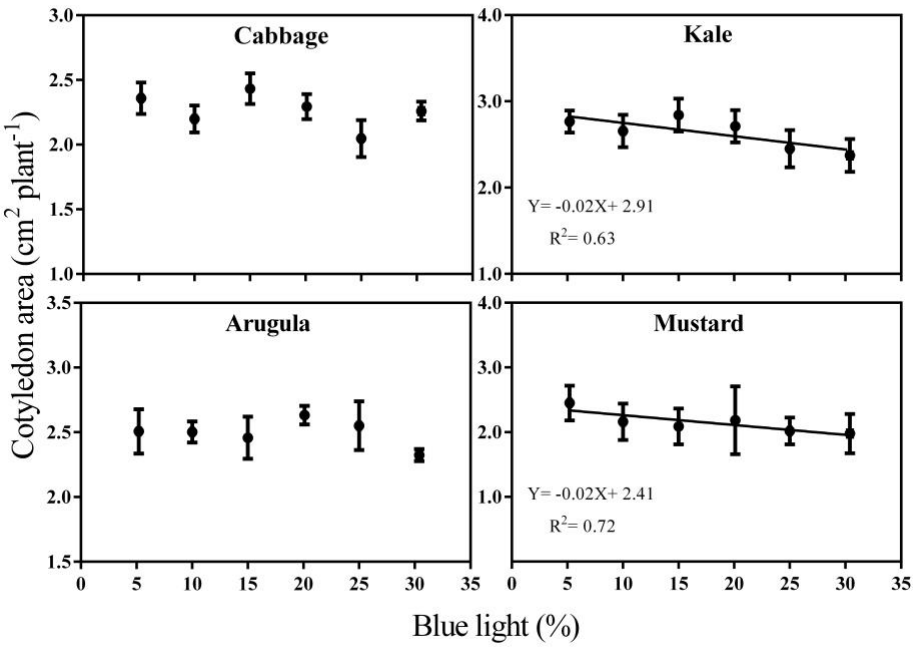


Figure 2.5. The response of cotyledon area to red and blue (B) LED lighting with varying B light percentage in four microgreens species. The total photosynthetic photon flux density (*PPFD*) of each lighting treatments was ≈ 300 µmol·m^{-2}·s^{-1}. Data are means ± SE of three separate experimental replicates. Regression lines are shown where the effects of B light percentage are significant at $P \leq 0.05$ and there are significant linear or non-linear regressions. The determination coefficient for the regression is shown as R^2.

2.3.3 Leaf color

With increasing B light percentage, cotyledons showed some red hues for green-leafed species and became darker red for red-leafed mustard (Fig. 2.6), and the average hue angle decreased from around 55˚ (yellow- orange) to 40˚ (orange) for cabbage, and from around 75˚ (dark green) to 60˚ (light green) for kale and arugula, and from around 10˚ (light red) to 0˚ (dark red) for mustard (Fig. 2.7).

Similarly, as B light percentage increased, the green% decreased linearly for cabbage, kale and arugula, which are green-leafed microgreens (Fig. 2.8). Also, the red% for red-leafed mustard also decreased linearly with increasing B light percentage (Fig. 2.7).

Figure 2.6. Cotyledon appearance of four microgreen species under red and blue (B) LED lighting with varying B light percentage. The total photosynthetic photon flux density (*PPFD*) of each lighting treatment was ≈ 300 $\mu mol \cdot m^{-2} \cdot s^{-1}$.

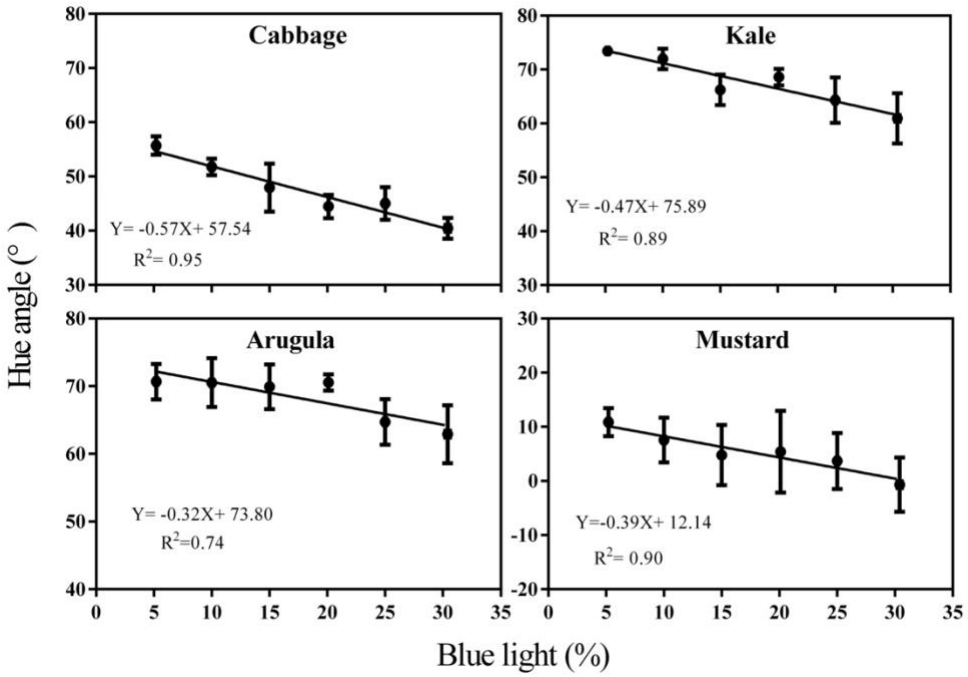


Figure 2.7. The response of hue angle in cotyledons to red and blue (B) LED lighting with varying B light percentage in four microgreens species. The total photosynthetic photon flux density (*PPFD*) of each lighting treatment was ≈ 300 µmol·m^{-2}·s^{-1}. Data are means ± SE of three separate experimental replicates. Regression lines are shown where the effects of B light percentage are significant at $P \leq 0.05$ and there are significant linear or non-linear regressions. The determination coefficient for the regression is shown as R^2.

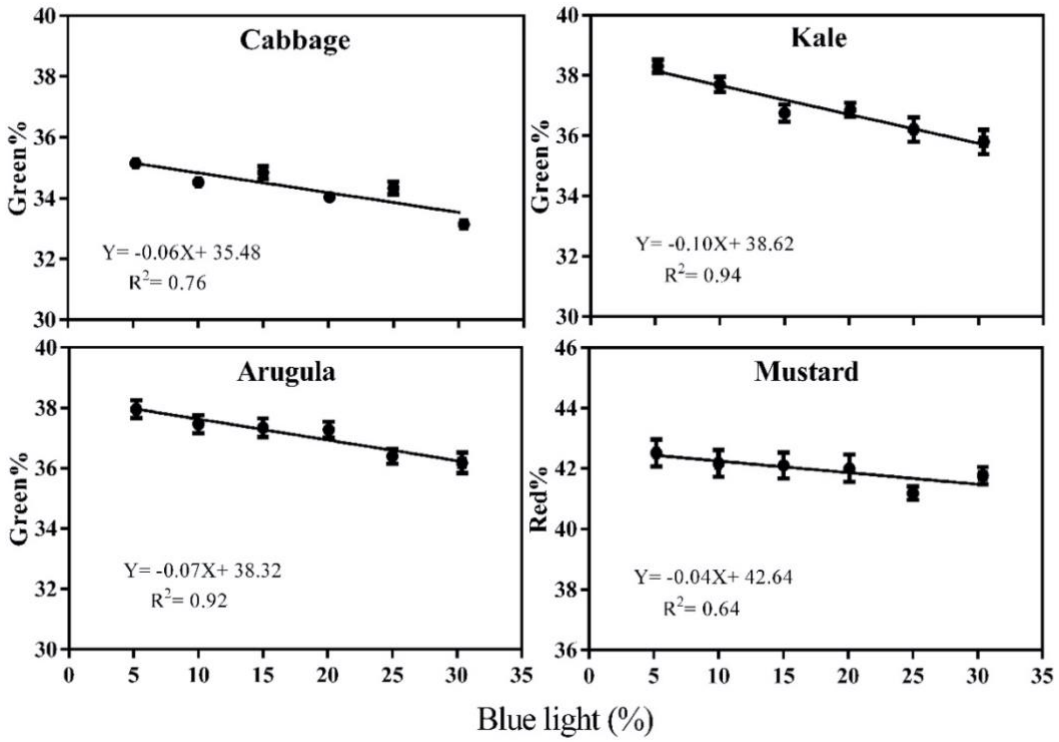


Figure 2.8. The response of green or red chromaticity (Green% or Red%) of cotyledons to red and blue (B) LED lighting with varying B light percentage in four microgreens species. The total photosynthetic photon flux density (*PPFD*) of each lighting treatment was ≈ 300 $\mu mol \cdot m^{-2} \cdot s^{-1}$. Data are means ± SE of three separate experimental replicates. Regression lines are shown where the effects of B light percentage are significant at $P \leq 0.05$ and there are significant linear or non-linear regressions. The determination coefficient for the regression is shown as R^2.

2.4 DISCUSSION

2.4.1 The effects of B light percentage on crop yield differ among species

For cabbage, in the present study, plants grown under 15% B light had the highest fresh yield. Similar response pattern was found in dry yield for this species. This suggested that this specific B light percentage (15%) was the most effective for enhancing yield. Nhut et al., (2002)

found a similar trend with banana plantlets *in vitro*, where both the plant shoot and root FW increased when B light increased from 10% to 20%, but decreased when B light increased from 20% to 30%. However, both the shoot and root FW of strawberry plantlets did not show difference under 10% and 20% B light, but decreased at 30% B light (Nhut et al., 2003). The different responses among species suggest that the effects of B light percentage on plant FW are species-specific.

In the present study, aside from cabbage, the other three microgreen species showed no differences in the fresh yield among all the treatments, indicating that the microgreen yields of these species were not affected by the variation of B light percentage within the tested range. Similar results have been reported in broccoli sprouts where the FW was similar under 5% and 20% B light of RB-LED lightings at a *PPFD* of 250 $\mu mol \cdot m^{-2} \cdot s^{-1}$ (Kopsell et al., 2014). In our study the dry yield of these three species was not affected by the variations of B light percentages either, which partly supported the similar fresh yield under different light treatments.

Previous studies on other plant species showed different results regarding the DW response to variation of B light percentage. The DW of tomato seedlings decreased with increasing B light from 10% to 30% and 50% under a total *PPFD* of 150 $\mu mol \cdot m^{-2} \cdot s^{-1}$ (Nanya et al., 2012). The DW of cucumber seedlings showed a linear decrease when B light increased from 10% to 75% under RB-LED lightings with a *PPFD* of 100 $\mu mol \cdot m^{-2} \cdot s^{-1}$ (Hernández and Kubota, 2016). The difference in the results between our and previous studies might be due to different species, growth periods and physiological stages. Unlike production of tomato and cucumber seedlings, most microgreens have a shorter growth period (around 12 d), and are harvested with two fully expanded cotyledons and one unfolded true leaf. It is a special growth stage

when they are transferring from heterotrophic growth that relies on seeds stored energy to autotrophic growth that relies on photosynthetic assimilates. In this case, the tested three microgreens, rather than the seedlings in other studies, might experience too short-time light treatments to induce significant different response in terms of photosynthesis.

2.4.2 The effects of B light percentage on appearance quality vary with traits and species

Plant color responses to B light differed between green- and red-leafed species in the present study. The green-leafed species (i.e., cabbage, kale and arugula) under higher percentages of B light showed some red or purple flushes on the surface of their leaves, to different degrees (Fig. 5). This was also supported by the linear decrease in cotyledon hue angle and green% with increasing percentage of B light. The increased red color formation in leaves could be caused by increased levels of anthocyanins under higher B light intensity (Carvalho and Folta, 2014; Owen and Lopez, 2015). Gonçalves et al. (2007) reported that hue angle was negatively correlated to anthocyanin content in cherries. Further, sole B light induced anthocyanin accumulation in many plant species including tomato (Giliberto, 2005), strawberry (Kadomura-Ishikawa et al., 2013), and sprouting kale (Carvalho and Folta, 2014). Moreover, red-leafed mustard showed a similar decreased response in hue angle as green-leafed species, indicating the red color of its leaves became darker with increasing B light percentage (Gonçalves et al., 2007). However, the red% of mustard leaves decreased linearly with increasing B light percentage. The contrasting responses between hue angle and red% for mustard might be due to the different calculation methods. Red% (or Green%) was calculated based on the percentage of total R, G, B value, while hue angle on the percentage of 255 (i.e., maximum value of R, B, or G). For mustard, its leaves showed visibly darker red color under

higher B light percentage, suggesting hue angle might be an accurate parameter to indicate actual color changes (Landschoot and Mancino, 2000). The reduced Red%, which contrasted with the increase in visible redness, was possibly due to a greater increase in G than R value under higher B light levels.

Plant size response to B light also varied with species in the present study. With the increase of B light percentage, there was a linear decrease in HL and cotyledon area for kale and mustard. Similar inhibition responses in stem or leaf have been reported when B light increased from 10% to 50% for tomato seedlings at a *PPFD* of 150 $\mu mol \cdot m^{-2} \cdot s^{-1}$ (Nanya et al., 2012), to 75% for cucumber seedlings at 100 $\mu mol \cdot m^{-2} \cdot s^{-1}$ (Hernández and Kubota, 2016), and to 30% for lettuce at 170 $\mu mol \cdot m^{-2} \cdot s^{-1}$ (Son and Oh, 2015). Also, reduced HL with increased B light percentage from 0.27% to 27.5% was found in tomato, cucumber, and pepper plants grown at a *PPFD* of either 200 or 500 $\mu mol \cdot m^{-2} \cdot s^{-1}$ (Snowden et al., 2016). The increased B light percentage might promote activity of cryptochrome, the B/UVA light photoreceptor (Lin and Shalitin, 2003). Active cryptochrome, besides phytochrome, is another inhibitor of elongation growth in plants (Neff and Van Volkenburgh, 1994). The linear decrease responses showed that the plants responded quantitatively to B light, which was also confirmed by Hogewoning et al., (2010). However, when B light percentage increased, there was no change in HL or cotyledon area for cabbage and arugula. The variation of responses among species might be due to their different sensitivities to B light. For example, Dougher and Bugbee (2001) found that lettuce was more sensitive to changes in B light percentage than wheat. Based on our results, it appeared that mustard and kale were more sensitive in plant size response to increasing B light than cabbage and arugula.

2.4.3 Optimization of B light percentage for microgreen production

To optimize B light percentage in RB-LED lighting for microgreen production, at least the following factors need to be considered. High yield is one of the most important factors for microgreen production, because it determines the profits that growers can make and thus the incentive to continue the business. Also, for microgreens, longer vs. shorter HL is easier for mechanical harvest. In addition, larger leaves with darker green or lighter red color are usually more attractive in appearance for green- or red-leafed microgreen species, although it may differ based on personal preferences. However, our results indicated that the above factors (e.g., yield and appearance quality) did not always move toward the desirable direction with increasing B light percentages for the tested species. It appears that even for the same crop species, the optimal light spectra requirement varies considerably between different physiological processes (Moe et al., 2006).

Taking into account the varied responses in microgreen yield and appearance quality to B light percentage, the optimal B light proportion should be determined to reach a balance of the consideration of yield and quality. For cabbage, the optimal B light percentage appears to be 15%, because at this level, the fresh yield was the highest and the leaf color was acceptable, while its HL and leaf area were not affected by B light percentage. B light percentage of 5% is recommended for arugula, kale, and mustard, because kale and mustard had the longest HL and largest leaves under 5% B light without any negative effects on fresh or dry yield.

The optimal B light percentage should help in developing RB-LED lighting ‘recipes’ for indoor production of microgreens to potentially achieve the maximum economic benefit for

growers. However, the results may only be applied to the same microgreen species, similar growth environment and cultivation method. Even for the same species, different photoperiod could also change the optimal B light percentage, since photoperiod could interact with B light level and affect plants growth (Atta et al., 2013). Also, if the same species is grown for a longer production period than microgreens, it may be necessary to take into consideration the difference in electric-energy-*PAR* conversion efficacy ($\mu mol \cdot J^{-1}$) between R and B LED of lighting. In this case, photosynthetic accumulation would make a substantial contribution to yield, so maximizing the electric-energy-to-plant-biomass conversion efficiency cannot be neglected to optimize B light percentage (Hernández and Kubota, 2014). Further research is also needed to investigate the effect of B light percentage in RB-LED lightings on other microgreen species, or in other growing systems.

2.5 CONCLUSION

In summary, the responses of crop yield and appearance quality of the four tested microgreens species to different B light percentage in RB-LED lighting varied with plant species and traits. Under the conditions of *PPFD* at ≈ 300 $\mu mol \cdot m^{-2} \cdot s^{-1}$ of RB-LED as sole light source, photoperiod at 16 h, and light/dark temperature at 20/16 °C, 15% B light was recommended for indoor microgreen production of cabbage, while 5% for the other three species. These results can potentially be used to help indoor microgreen growers to optimize their production.

CHAPTER THREE

THE INCREASED PROPORTION OF BLUE LIGHT FROM LIGHT-EMITTING DIODES AFFECTS THE PHYTOCHEMICAL COMPOSITION OF MICROGREENS

ABSTRACT

Microgreens are specialty vegetables that contain human health-promoting phytochemicals. Typically, microgreens are cultivated in controlled environments under R and B LEDs. However, the impact of varying the proportion of these light qualities on the composition of diverse phytochemicals in indoor-grown microgreens is unclear. To address this problem, the levels of chlorophylls, carotenoids, ascorbates, phenolics, anthocyanins, and nitrate were examined in arugula (*Eruca sativa* L.), 'Red Russian' kale (*Brassica napus* L. subsp. *napus* var. *pabularia* [DC.] Alef.), 'Mizuna' mustard (*Brassica juncea* L.) and red cabbage (*Brassica oleracea* L. var. *capitata* f. *rubra*) microgreens following cultivation under LEDs supplying varying proportions of B light (5% to 30%), together with R light (95% to 70%). Varying the proportion of B light did not affect the extractable levels of total chlorophyll, total carotenoids, or nitrate in all four microgreen species. Generally, the levels of reduced and total ascorbate were greatest in arugula, kale and mustard microgreens at 20% B light, and a minor decrease was apparent at 30% B light; these metabolite profiles were not impacted by B light percentage in red cabbage. Kale and mustard accumulated more total phenolics at 30% B light, than all other B light regimens, whereas this phytochemical attribute was unaffected in arugula and red cabbage. The total anthocyanin concentration increased proportionally with the percentage of supplied B light up to 30% in all microgreens, with exception of mustard. Taken together, our research showed that the 20% B light supplied from LED arrays is ideal to achieve optimal levels of both reduced and total

ascorbate in all microgreens except red cabbage, whereas 30% B light promotes the greatest accumulation of total anthocyanin in indoor-grown *Brassicaceae* microgreens, with exception of mustard.

3.1 INTRODUCTION

Microgreens are a group of specialty crops that are normally harvested when both cotyledons have expanded and the first true leaf starts to emerge (Treadwell et al., 2016). Microgreens are a rich source of health-promoting phytochemicals as compared to their corresponding mature plants (Xiao et al., 2012). Phytochemicals that are present in microgreens, specifically in those of the *Brassicaceae* family, include ascorbate (i.e., vitamin C), chlorophylls, carotenoids, phenolics, and anthocyanins (Xiao et al., 2019). Although light harvesting pigments such as chlorophylls are not well-known for the human health benefits, the consumption of the other aforementioned phytochemicals can potentially reduce the risk of chronic and degenerative diseases (Zhang et al., 2015). Carotenoids include carotenes and xanthophylls that function as accessory photosynthetic pigments in plants, some of which are converted into vitamin A in humans which is crucial for vision, or as antioxidants that can prevent cancer and other chronic diseases (Rao and Rao, 2007; Thoma et al. 2020). Another well-known plant-derived antioxidant is ascorbate. In humans, ascorbate deficiency is linked to scurvy, and dietary ascorbate regulates collagen formation, iron uptake and is involved in epigenetic control of various diseases (Macknight et al. 2017). Phenolic compounds that occur in plants including simple aromatic compounds such as hydroxycinnamic acids and complex polyphenols like anthocyanin pigments. Apart from their antioxidant benefits, some of these phenolic molecules have anti-inflammatory effects in human (Zhang and Tsao, 2016). In

addition, microgreens contain lower levels of nitrate as compared to mature leafy vegetables (Pinto et al., 2015). From a human health standpoint, the effect of dietary nitrate has been the subject of much debate. There is evidence that nitrate derived from vegetables in the diet can provide cardioprotective benefits, including the capacity to lower blood pressure (Machha and Schechter, 2012). There is some concern that an excess of food-derived nitrate can be problematic as it is sequentially converted into nitrite and nitrosamine in the body; the accumulation of the latter is associated with an increased risk of certain types of cancer (Mensinga et al., 2003; Bian et al., 2015).

Microgreens can be grown in indoor environments with electrical lighting as a sole light source, which circumvents the restrictions that ambient light has on plant growth, such as biomass accumulation (Darko et al., 2014). LED technology has many advantages over traditional light sources (e.g. high-pressure sodium light and fluorescent light) including energy efficiency, low heat output, long operating life-time and is less environmentally costly (Bourget, 2008). Moreover, LEDs afford the opportunity to modulate spectral composition in order to optimize the morphology and phytochemical distribution of indoor grown plants (Bian et al., 2015; Yeh and Chung, 2009). It is known that B light (400–500 nm) and R light (600–700 nm) are readily absorbed by leaves and utilized for photosynthesis (McCree, 1971). R light supplemented with B light increased photosynthetic capacity and biomass accumulation in plant seedlings (Hogewoning et al., 2010; Tanaka et al., 1998). The combination of B and R LEDs is widely used for horticultural crop production, including microgreens (Jones-Baumgardt et al., 2019; Massa et al., 2008; Ying et al., 2020).

Typically, the *PPFD* that is supplied to leafy green vegetables that are cultivated indoors can be as high as 300 $\mu mol \cdot m^{-2} \cdot s^{-1}$ (Thoma et al. 2020). For *Brassicaceae* microgreens grown under B and R LEDs, *PPFD* of 300 to 400 $\mu mol \cdot m^{-2} \cdot s^{-1}$ are optimal for yield and aesthetic quality (Jones-Baumgardt et al., 2019). However, high B:R light ratios are known to decrease the growth and yield of herbs (Dou et al., 2017). A recent study found that LEDs supplying R:B light ratios of 95% R:5% B to 85% R:15% B are optimal for hypocotyl length, and cotyledon area in kale and mustard microgreens, whereas a reduction in these morphological attributes is apparent at higher B light percentages (Ying et al., 2020). In addition, microgreens amass more reddish than green surface coloration when the proportion of B light in the LED array is increased from 5% to 30% (Ying et al., 2020). These morphological changes may be associated with modifications in plant vitamins and secondary metabolites under increasing proportion of B light supplied by LEDs and potentially affect the nutritional quality.

Light quality has a pronounced effect on the levels of phytochemicals that accumulate within plants under controlled environments (Bian et al., 2015). Previous studies indicate that the phytochemical profiles vary with the proportion of B light supplied by LEDs, albeit in a manner that is plant species and/or developmental stage dependent, including in their microgreen forms. For example, the amount of chlorophyll in cucumber (*Cucumis sativus* L.) leaves elevated with an increase in B light proportion from 0% to 50% supplied from R:B LEDs (Hogewoning et al., 2010). In contrast, there was no difference in the chlorophyll concentration of tomato (*Solanum lycopersicum* L.), salvia (*Salvia splendens* Sellow ex Schult) or impatiens (*Impatiens walleriana* Hook.f.) seedlings cultivated under LEDs with varying B light in the range of 6% to 50% (Wollaeger and Runkle, 2015). Increasing the percentage of B light from 0% to

33% enhanced the total carotenoid concentration in beet (*Beta vulgaris* L.) microgreens, but not in parsley (*Petroselinum crispum* (Mill.) Fuss) or mustard (*Brassica juncea* (L.) Czern.) microgreens (Samuolienė et al., 2017). Similarly, the proportion of B light had no effect on ascorbate levels in rapeseed (*Brassica napus* L.) plantlets (Li et al., 2013), whereas ascorbate tended to be present at higher levels in lettuce (*Lactuca sativa* L.) cultivated under LEDs supplying 75% B:25% R light relative to those delivering 25% B:75% R and 50% B: 50% R light (Zha et al., 2020). The impact of B light on ascorbate metabolism has not been widely explored in microgreens. It is worth mentioning that the Li et al. (2013) study assessed ascorbate using a spectrophotometric analysis of plantlet extracts. This is not ideal, as spectrophotometric analysis of plant ascorbate requires higher specificity based upon ascorbate oxidase coupled assays (Queval and Noctor, 2007), or spectrophotometric detection following HPLC separation (Flaherty et al., 2018). Increased levels of total phenolic compounds in lettuce elevated as the proportion of B light increased 0% to 47% (Son and Oh, 2013). Although the alteration of anthocyanin concentrations in microgreens in response to increasing proportion of B light is not well understood, anthocyanin levels were enhanced in baby red lettuce cultivated under cool white fluorescent lamps supplemented with B LEDs (Li and Kubota, 2009). In addition, anthocyanin accumulated in the leaves of mature pepper (*Capsicum annuum* L.) plants cultivated under intermittent illumination with high percentage B light (Hoffmann et al., 2016). Although the aforementioned studies have looked at the impact of B light on select phytochemicals, a comprehensive analysis of the impact of this light quality on simultaneous alterations of photosynthetic pigments, ascorbate metabolites, phenolics and anthocyanins, as well as nitrate in *Brassicaceae* microgreens has not been explored. Herein, we investigated the

effect of sole source LED arrays supplying varying proportions of B light, specifically 5% to 30% with the remainder consisting of R light, on the levels of total chlorophyll and its individual components, total carotenoid, total phenolic, total anthocyanin, reduced ascorbate, dehydroascorbate, and nitrate in arugula, kale, mustard and red cabbage microgreens.

3.2 MATERIALS AND METHODS

3.2.1 Experimental materials and design

The experiment was conducted in a controlled environment walk-in growth chamber (7.3 m × 4.0 m × 2.5 m; length × width × height) at the University of Guelph. Details related to the experimental design and environmental growth conditions are described in a previous paper (Ying et al., 2020). Briefly, seeds of arugula (*Eruca sativa* L.), 'Red Russian' kale (*Brassica napus* L. subsp. *napus* var. *pabularia* [DC.] Alef.), 'Mizuna' mustard (*Brassica juncea* L.), and red cabbage (*Brassica oleracea* L. var. *capitata* f. *Rubra*) were separately sown on organic substrates supplied by Greenbelt Microgreens Inc. (Lynden, ON, Canada) in fibre trays (23.5 cm × 48.5 cm × 3.5 cm). After watering, the sown microgreen trays were randomly placed in six separate compartments within the growth chamber under different lighting treatments. Opaque white vertical blinds separated the light quality treatment compartments within the growth chamber. Each lighting treatment compartment contained two Heliospectra LED arrays (LX602C, Heliospectra AB, Gothenburg, Sweden) that were positioned 36 cm apart in the center of the plot area, and 56.5 cm above the substrate in the seed trays. The experiment was comprised of six light quality treatments of varying proportions of B (445 nm peak) and R (660 nm peak) light supplied from the aforementioned LED arrays. These six LED regimens were as follows: 5% B light and 95% R light; 10% B light and 90% R light; 15% B light and 85% R light;

20% B light and 80% R light; 25% B light and 75% R light; and 30% B light and 70% R light. All lighting treatments had a fixed *PPFD* of 300 $\mu mol \cdot m^{-2} \cdot s^{-1}$ and were applied for a 16 h-photoperiod. For each light quality treatment, *PPFD* and spectral distribution were measured at 25 separate canopy level positions as described previously (Ying et al. 2020). For simplicity, from henceforth all LED quality treatments are given as the percentage of supplied B light. For each lighting treatment, there were four trays of sown seeds, with each tray containing only one of the aforementioned species. The experiment was a randomized complete block design with six light treatments within a single controlled environment chamber. The temperature in the walk-in controlled environment chamber was set and maintained at 21 °C during the light period (10 a.m. to 2 a.m.) and 17 °C during the dark period. The relative humidity was maintained at approximately 80% over the course of the experiment. The temperature and relative humidity were monitored by an automated system (Argus Controls Systems Ltd., Surrey, BC, Canada). All growing substrates were top irrigated at least once each day using well water until the appearance of visible drainage. A total of three separate light quality experiments were performed.

3.2.2 Tissue sampling for phytochemical analyses

For each microgreen species and light quality treatment within each experiment, three subsamples (approximately 5 g per subsample) of above-ground plant parts, including cotyledons, stems and first true leaves were collected 10 d after sowing for kale and red cabbage, and 11 d after sowing for arugula and mustard. The harvested microgreens were immediately flash frozen in liquid N_2 and pulverized with a pre-chilled mortar and pestle. The flash frozen microgreen powders were stored at -80 °C for up to 8 months prior to

phytochemical analyses. Unless otherwise mentioned, all chemicals required were purchased from Sigma-Aldrich Inc. (Oakville, ON, Canada). Glacial acetic acid, methanol and *ortho*-phoshporic acid were obtained from Thermo-Fisher Scientific (Mississauga, ON, Canada).

3.2.2.1 Chlorophyll and carotenoids

For the analysis of chlorophyll and carotenoids from each microgreen LED regimen treatment replicate, cryogenic microgreen powder (20 mg) was transferred to a pre-chilled Eppendorf tube and re-suspended in 1 mL of ice-cold 100% methanol. The sample was vortexed for 1 min, incubated on ice under darkness for approximately 10 min, followed by a second mixing by vortexing prior to centrifugation at 21000 *g* for 5 min at 4°C. The supernatant was collected and serial dilutions (up to three times) in a final volume of 200 µL were prepared in 100% ice-cold methanol. The methanolic extract and its dilutions were transferred to a 96-well microplate reader (SpectraMax 384 Plus; Molecular Devices, Sunnyvale, CA, USA) and absorbance readings were taken at 665, 652 and 476 nm for chlorophyll *a*, chlorophyll *b* and carotenoids, respectively. The chlorophyll *a*, chlorophyll *b* and total carotenoid concentrations were calculated using the equations for methanolic solutions as described in Lichtenthaler and Buschmann (2001).

3.2.2.2 Ascorbate metabolites

An established method from Flaherty et al. (2018) was used to assess reduced ascorbate, dehydroascorbate and total ascorbate concentrations. These metabolites where extracted from cryogenically frozen microgreen powders (200 mg) after grinding the tissue in an ice-cold mortar and pestle with 500 µL of 6% (w/v) *meta*-phosphoric acid in the presence of acid-washed silica sand, and then centrifuged at 13000 *g* for 10 min at 4 ºC. The supernatant

was filtered through a 0.45 μm polytetrafluoroethylene filter (13 mm diameter; Mandel Scientific Inc, Guelph, Ontario, Canada). The preparation containing reduced ascorbate was analyzed by HPLC as described below. For the analysis of total ascorbate, the supernatant was subjected to a chemical reduction to promote the conversion of dehydroascorbate to reduced ascorbate. For this, a 50 µL aliquot of the supernatant was combined with 25 µL of 400 mM dithiothreitol (DTT) in 2 M Tris base. After incubating for 15 min at 24 ºC, 25 µL of 8.5% (v/v) *ortho*-phosphoric acid was added to terminate the reduction step.

The total and reduced ascorbate preparations were analyzed by HPLC. For this 1 µL of the non-DTT treated extract (reduced ascorbate) and 2 µL of the DTT-treated extract (total ascorbate) were separately analyzed via a HPLC procedure. The aforementioned extracts were injected on a Restek Ultra Aqueous C18 column (150 x 4.6 mm, 5 µm particle; Chromatographics Specialties Inc, ON, Canada) attached to an Agilent 1200 HPLC (Agilent Technologies, Mississauga, ON, Canada) system, and thermostatted to 20 °C. Ascorbate was eluted isocratically with 20 mM *ortho*-phosphoric acid at a flow rate of 1 $mL \cdot min^{-1}$ over 10 min. The absorbance of the eluate was monitored at 254 nm and ascorbate peaks were compared to a known range of authentic L-ascorbate (Sigma-Aldrich Inc.) standard for quantification of the reduced ascorbate in the DTT-free extract and the total ascorbate in the DTT-treated extract. The dehydroascorbate concentration was calculated by subtracting the ascorbate detected in the DTT-free extract from that apparent in the DTT-treated extract. The levels of ascorbate in both DTT and non-DTT containing extracts were corrected by assessing the recovery of a known amount of authentic reduced ascorbate (377 to 778 nmol) that was added to a representative

duplicate extract. The HPLC analysis revealed that the recovery of the reduced ascorbate that was spiked into the ascorbate extract was in the range of 70 to 97%.

3.2.2.3 Total Phenolic

The total phenolic concentration was evaluated according to the procedure described by Ainsworth and Gillespie (2007) with some modifications. Approximately 20 mg of frozen microgreen powder was transferred to a 2 mL Eppendorf tube, resuspended in 1 mL ice-cold 100% methanol and vortexed twice for 1 min. The sample was then centrifuged at 13000 *g* for 5 min at 4 ºC. A 25 µL aliquot of the supernatant and dilutions prepared in 100% methanol were separately dispended into wells of a 96-well microplate. Thereafter, 125 µL of 10% (v/v) Folin-Ciocalteau reagent was added to each well, and the plate was incubated at room temperature for 10 min, followed by the addition of 125 µL of 7.5% (w/v) Na_2CO_3. The absorbance was measured at 765 nm using a SpectraMax 384 Plus microplate reader and total phenolic concentration was compared to an authentic gallic acid standard curve ranging from 0.018 to 0.6 $mg \cdot mL^{-1}$.

3.2.2.4 Total Anthocyanin

For each microgreen and its light quality treatment replicate subsample, anthocyanins were extracted with acidified methanol according to the method of Roepke and Bozzo (2015). For the extraction process, 100 mg of cryogenically frozen microgreen powder was combined with 500 µL of methanol: acetic acid: Milli-Q water (9:1:10, v/v/v). The homogenate was vortexed for 1 min, agitated on a nutator (Adams™ Nutator; Becton, Dickinson and Company, Franklin Lakes, NJ, USA) for 20 min and then centrifuged at 16000 *g* for 10 min. The supernatant was collected and the residue was re-extracted twice as described above. The supernatants from each

successive extraction were pooled, and partitioned against an equivalent volume of chloroform for the removal of chlorophyll. The 1.5 mL acidified methanol phase was collected and taken to dryness in a speedvac (Savant SC100 Speed Vac Concentrator; Thermo Fisher Scientific). The anthocyanin residue was dissolved in 200 µL of methanol containing 0.1% HCL (v/v). Thereafter, the absorption was measured at 530 nm with a SpectraMax 384 Plus microplate reader. The total anthocyanin content was expressed as cyanidin 3-*O*-glucoside equivalents using a molar absorptivity (ε) of 34300 $L \cdot mol^{-1} \cdot cm^{-1}$ (Siegelman and Hendricks, 1958).

3.2.2.5 Nitrate

The nitrate concentration in microgreens was analyzed using a microplate spectrophotometer method developed by Hachiya and Okamoto (2017). For each microgreen LED light quality treatment experimental replicate, 50 mg of frozen microgreen powder was transferred to a microcentrifuge tube and combined with 500 µL Milli-Q water, followed by incubation in a boiling water bath for 20 min. After cooling down to room temperature, the sample was centrifuged at 20400 *g* for 10 min at room temperature. A 10 µL aliquot of the extract was mixed with 40 µL of 0.05% (w/v) salicylic acid in sulfuric acid, followed by incubation at room temperature for 20 min. Thereafter, 1 mL of 8% (w/v) NaOH was added to each sample. The absorbance was measured at 410 nm with a SpectraMax 384 Plus microplate reader and compared to a nitrate standard curve ranging from 12.65 to 1600 $\mu g \cdot mL^{-1}$. For each sample, non-specific absorbance was estimated by incubating the aqueous extract with 40 µL of sulfuric acid. In order to determine the amount of nitrate per microgreen sample, the non-specific absorbance was subtracted from absorbance of an assay performed in the presence of salicylic acid.

3.2.3 Statistical analyses

The relationship between alterations in phytochemical concentrations in each microgreen as a function of B light percentage treatment were determined with regression analyses. To this end, linear and quadratic regressions for phytochemical data corresponding to experimental treatment replicates and their subsamples were established with SPSS software (Version 25.0, IBM, New York, USA). Data were analyzed for normality of residuals and homoscedasticity by using the Shapiro-Wilk and Levene test, respectively. Where applicable, heteroscedasticity of the data was verified with residual plots.

3.3 RESULTS

3.3.1 Chlorophyll and carotenoid concentrations

There was no effect of altering the percentage of B light supplied from LEDs delivering a *PPFD* of 300 $\mu mol \cdot m^{-2} \cdot s^{-1}$ on the concentrations of total chlorophyll and chlorophyll *a* for all four microgreen species (Fig. 1). The average total chlorophyll content across all six B light treatments were 0.71, 0.51, 0.62 and 0.69 $mg \cdot g\ FW^{-1}$ for arugula, kale, mustard and red cabbage, respectively. Similarly, there was no effect of the B light percentage supplied from LEDs on chlorophyll *b* with the exception of mustard. To this end, the chlorophyll *b* concentration in mustard microgreens cultivated under 30% B light was 14 % smaller than those cultivated under 5% B light (Fig. 3.1). The total carotenoid concentration was similar across all LED light treatments, irrespective of species (Fig. 3.2). The average concentration of this phytochemical was 0.1 $mg \cdot g\ FW^{-1}$ across all four microgreens and their light quality treatments.

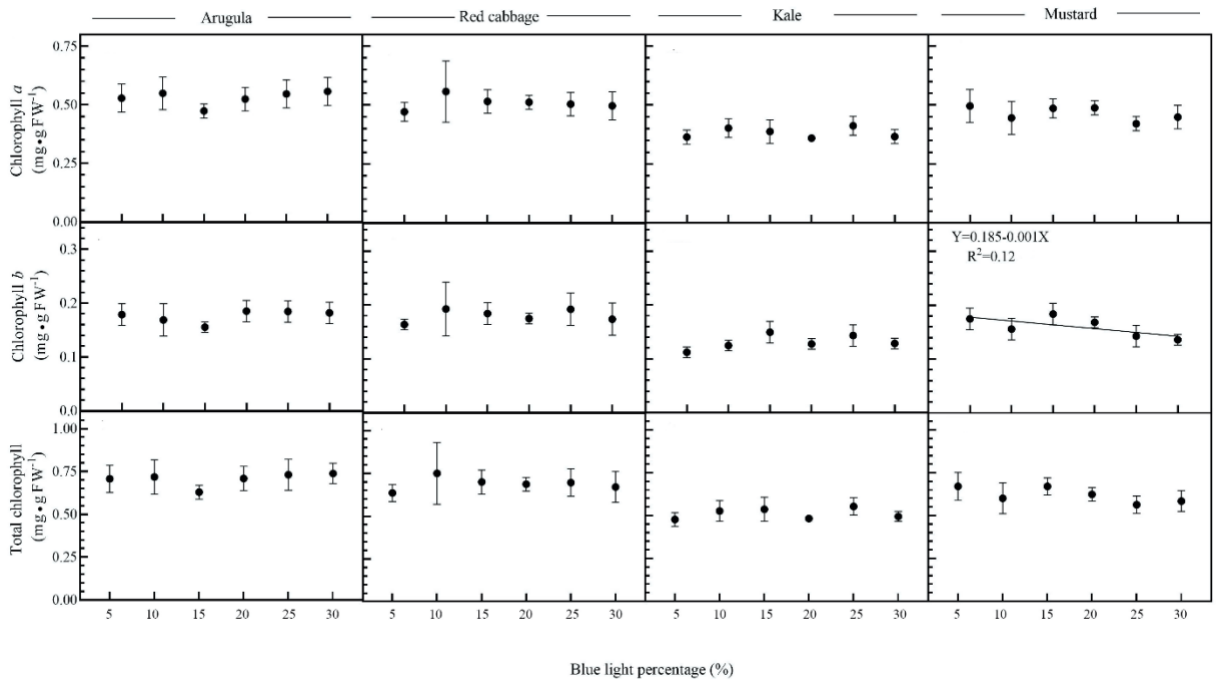


Figure 3.1. The impact of light quality on chlorophyll *a*, chlorophyll *b* and total chlorophyll concentrations of arugula (*Eruca sativa* L.), 'Red Russian' kale (*Brassica napus* L. subsp. *napus* var. *pabularia* [DC.] Alef.), 'Mizuna' mustard (*Brassica juncea* L.) and red cabbage (*Brassica oleracea* L. var. *capitata* f. *rubra*). In all cases, microgreens were cultivated under a constant *PPFD* of 300 $\mu mol \cdot m^{-2} \cdot s^{-1}$ with varying percentages of blue (B) light. Data represent the mean ± SE of three separate experiments. A linear regression line represented by a solid black line is shown where the effect of B light percentage was significant at $P \leq 0.05$; R^2 represents the corresponding determination coefficient.

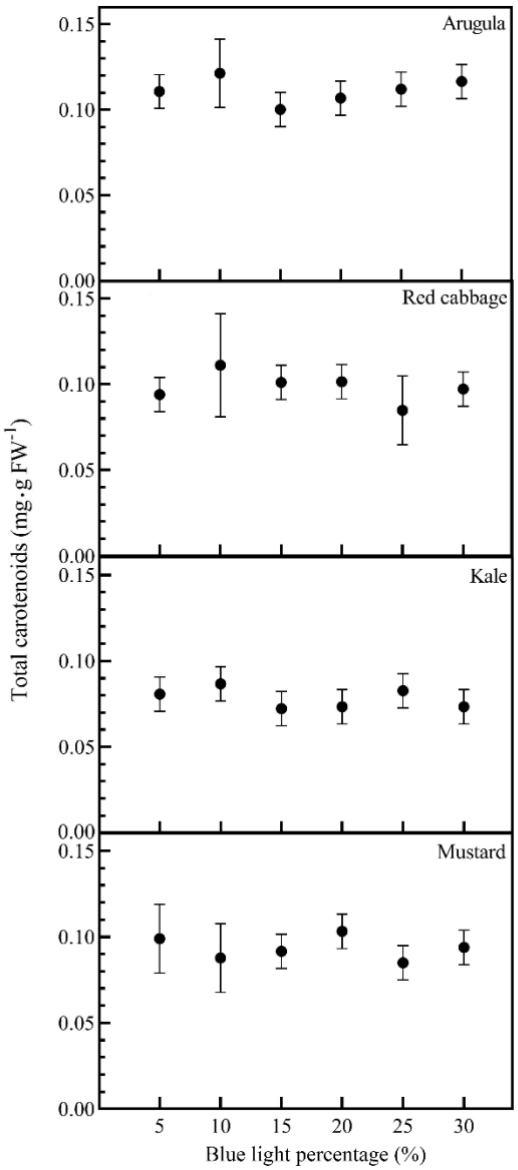


Figure 3.2. The impact of light quality on total carotenoid concentrations of arugula (*Eruca sativa* L.), 'Red Russian' kale (*Brassica napus* L. subsp. *napus* var. *pabularia* [DC.] Alef.), 'Mizuna' mustard (*Brassica juncea* L.) and red cabbage (*Brassica oleracea* L. var. *capitata* f. *rubra*). In all cases, microgreens were cultivated under a constant *PPFD* of 300 µmol·m^{-2}·s^{-1} with varying percentages of B light. Data represent the mean ± SE of three separate experiments. For each microgreen species, data were analyzed using a regression analysis, but there was no significant effect of B light percentage on total carotenoid concentrations.

3.3.2 Ascorbate metabolite concentrations

The effect of altering the B light percentage supplied from sole source LEDs on ascorbate metabolite composition in microgreens varied with species (Fig. 3.3). For red cabbage, the reduced ascorbate concentration was comparable across all light quality treatments. For the other microgreens, the concentrations of reduced and total ascorbate increased 13% to 29% in microgreens cultivated under 20% B light relative to 5% B light. Thereafter, 9% to 11% smaller concentrations of reduced ascorbate, and 7% to 15% smaller total ascorbate levels were apparent at 30% B light relative to these maximal levels (Fig. 3.3). Dehydroascorbate levels were unaffected by the percentage of B light with exception of arugula, which was 200% to 344% greater under B light percentages of 15 to 20% relative to smallest levels detected at 5% and 30% B light. Dehydroascorbate accounted for 3% to 13% of the total ascorbate in the tested microgreen species.

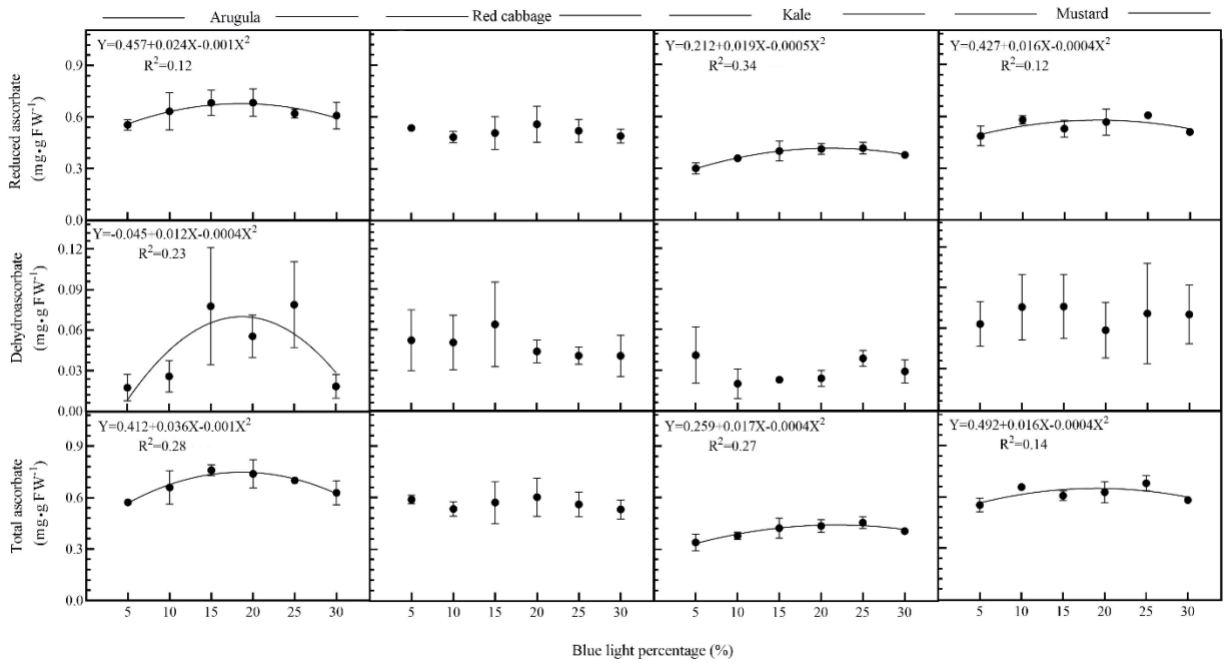


Figure 3.3. The impact of light quality on reduced ascorbate, dehydroascorbate and total ascorbate concentrations of arugula (*Eruca sativa* L.), 'Red Russian' kale (*Brassica napus* L. subsp. *napus* var. *pabularia* [DC.] Alef.), 'Mizuna' mustard (*Brassica juncea* L.) and red cabbage (*Brassica oleracea* L. var. *capitata* f. *rubra*). In all cases, microgreens were cultivated under a constant *PPFD* of 300 $\mu mol \cdot m^{-2} \cdot s^{-1}$ with varying percentages of blue (B) light. Data represent the mean ± SE of three separate experiments. A nonlinear regression line represented by a solid black line is shown where the effect of B light percentage was significant at $P \leq 0.05$; R^2 represents the corresponding determination coefficient.

3.3.3 Total phenolic and total anthocyanin concentrations

The total phenolic concentration of microgreens was affected by light quality, although in a species-dependent manner (Fig. 3.4). For kale, an increased proportion of B light culminated in more total phenolics; for example, cultivation under 30 % B light yielded 18% greater total phenolic concentration than that apparent under 5% B light. Similarly, the concentration of total phenolics in mustard microgreens increased linearly with elevated proportions of B light; levels detected at 30% B light were 24% greater than those apparent in the lowest B light percentage treatment. The concentrations of total phenolics in red cabbage and arugula were not impacted by varying the B light percentage supplied from LED arrays during controlled environment cultivation.

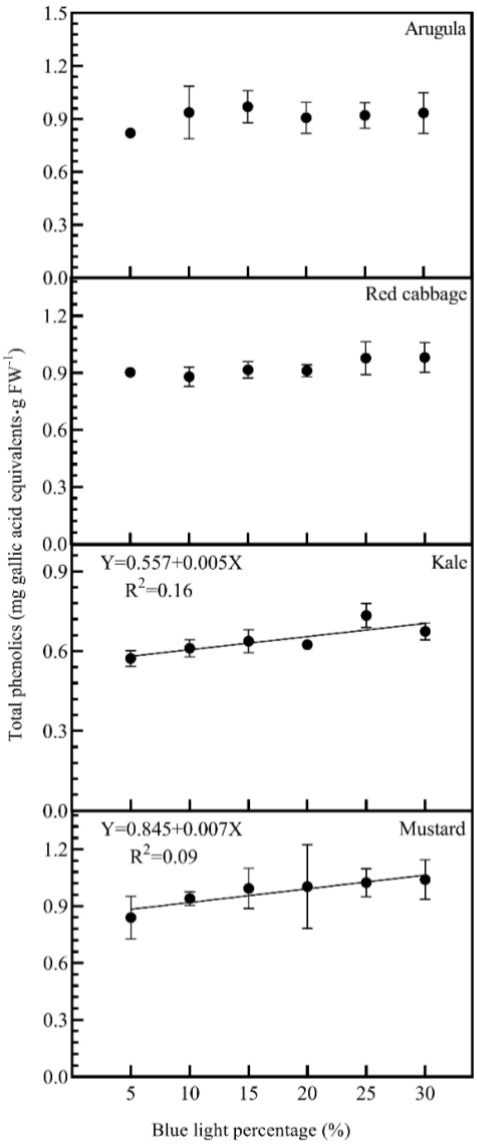


Figure 3.4. The impact of light quality on total phenolic concentrations of arugula (*Eruca sativa* L.), 'Red Russian' kale (*Brassica napus* L. subsp. *napus* var. *pabularia* [DC.] Alef.), 'Mizuna' mustard (*Brassica juncea* L.) and red cabbage (*Brassica oleracea* L. var. *capitata* f. *rubra*). In all cases, microgreens were cultivated under a constant *PPFD* of 300 µmol·m^{-2}·s^{-1} with varying percentages of blue light. Data represent the mean ± SE of three separate experiments. A linear regression line represented by a solid black line is shown where the effect of B light percentage was significant at $P \leq 0.05$; R^2 represents the corresponding determination coefficient.

The total anthocyanin concentration was enhanced in arugula, kale and red cabbage in response to an increased proportion of B light (Fig. 3.5). In arugula, kale and red cabbage, the total anthocyanin levels were respectively 70%, 65% and 75% greater in microgreens cultivated under 30% B light than at the 5% B light treatment. Total anthocyanin concentrations in mustard were similar for all LED treatments, and were unaffected by the percentage of B light. At its maximum, the absolute anthocyanin concentration in mustard microgreens was 1.7- to 18.6-fold that of the highest levels detected in the other three microgreens.

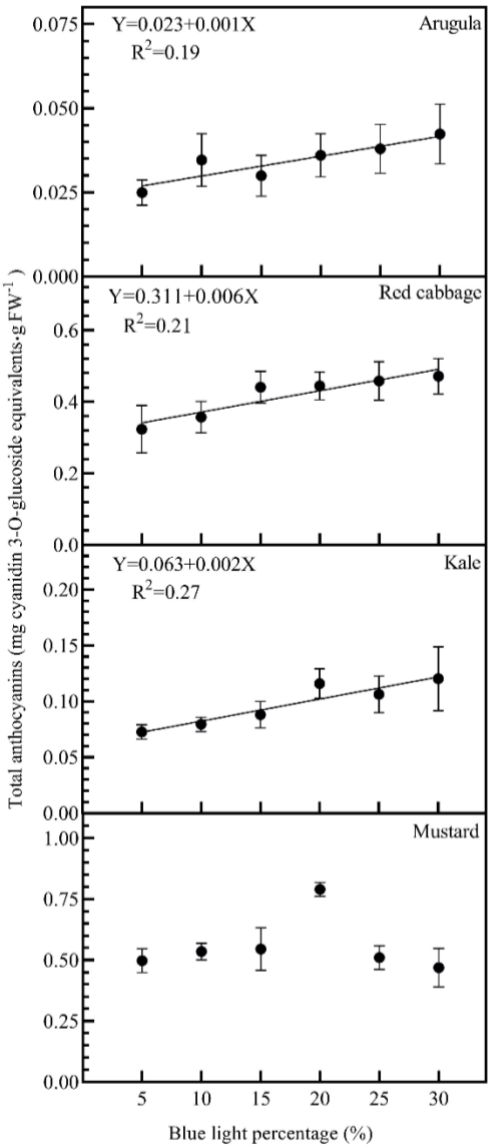


Figure 3.5. The impact of light quality on total anthocyanin concentrations of arugula (*Eruca sativa* L.), 'Red Russian' kale (*Brassica napus* L. subsp. *napus* var. *pabularia* [DC.] Alef.), 'Mizuna' mustard (*Brassica juncea* L.) and red cabbage (*Brassica oleracea* L. var. *capitata* f. *rubra*). In all cases, microgreens were cultivated under a constant *PPFD* of 300 µmol·m^{-2}·s^{-1} with varying percentages of B light. Data represent the mean ± SE of three separate experiments. A linear regression line represented by a solid black line is shown where the effect of B light percentage was significant at $P \leq 0.05$; R^2 represents the corresponding determination coefficient.

3.3.4 Nitrate concentration

The concentration of extractable nitrate was unaffected by the percentage of B light supplied by the LED array, regardless of microgreen species (Table 3.1). The nitrate levels detected across all microgreens and their LED light quality treatments was between 0.10 to 0.72 $mg \cdot g^{-1}$ FW. The average level was greatest in red cabbage and the least prevalent in arugula, with respect to extractable nitrate concentration.

Table 3.1. The impact of light quality on total nitrate concentrations of arugula (*Eruca sativa* L.), 'Red Russian' kale (*Brassica napus* L. subsp. *napus* var. *pabularia* [DC.] Alef.), 'Mizuna' mustard (*Brassica juncea* L.) and red cabbage (*Brassica oleracea* L. var. *capitata* f. *rubra*).

Blue light percentage [z]	Nitrate ($mg \cdot g\ FW^{-1}$) [y]			
	Red cabbage	Kale	Arugula	Mustard
5%	0.53 ± 0.30	0.34 ± 0.21	0.41 ± 0.38	0.12 ± 0.05
10%	0.47 ± 0.28	0.39 ± 0.33	0.29 ± 0.26	0.27 ± 0.10
15%	0.60 ± 0.30	0.38 ± 0.26	0.41 ± 0.37	0.15 ± 0.05
20%	0.44 ± 0.28	0.26 ± 0.12	0.41 ± 0.37	0.10 ± 0.05
25%	0.72 ± 0.41	0.29 ± 0.17	0.30 ± 0.24	0.22 ± 0.11
30%	0.61 ± 0.33	0.40 ± 0.20	0.31 ± 0.25	0.16 ± 0.07

[z] Microgreens were cultivated under a *PPFD* of 300 $\mu mol \cdot m^{-2} \cdot s^{-1}$ with varying proportions of red and blue light.

[y] Data represent the mean ± SE of three separate experiments.

3.4 DISCUSSION

3.4.1 Effect of varying B light percentages in R:B LED lighting on the concentrations of total chlorophylls, carotenoids, and nitrate in microgreens

The percentage of B light supplied by LEDs did not affect the total chlorophyll in the four microgreen species. The impact of B light on chlorophyll levels in plants during early development seems to be species-dependent. Similarly, chlorophyll levels in impatiens, salvia and tomato were unaffected by B light percentages in the range of 6% to 50% supplied by R:B LEDs (Wollaeger and Runkle, 2015), or in spinach (*Spinacia oleracea* L.) when cultivated at B light greater than 10% (Matsuda et al., 2007). Conversely, cucumber seedlings accumulated more chlorophyll when B light increased from 0% to 50% in B:R LEDs (Hogewoning et al., 2010). In lettuce, total chlorophyll was cultivar-dependent as the levels of this photosynthetic pigment increased in the red-leaf lettuce cultivar 'Sunmang' when the proportion of B light increased from 10% to 20%; by comparison, the green-leaf lettuce cultivar 'Grand Rapid TBR' was not affected in the same manner (Son and Oh, 2015). Chlorophyll biosynthesis is regulated by B light photoreceptors (i.e., cryptochrome), as well as phytochrome proteins that interact with R and FR light (Liu et al. 2017; Zheng et al., 2019). In our study, the increased supply of B light to indoor grown microgreens corresponded with a decreased supply of R light from sole source LEDs. The reduction in R light availability may have been instrumental in the lack of an effect on chlorophyll production in all four microgreen species investigated here, as there is a precedence that R light of 660 nm promotes the activation of phytochrome B, and consequently the involvement of this protein in chlorophyll biosynthesis (Kreslavski et al. 2018). Thus, lowering the proportion of R light at the expense of enhancing that of B light may have

reduced the activation of phytochrome-mediated regulation of chlorophyll biosynthesis in the *Brassicaceae* microgreens investigated in this study.

Total carotenoid concentration was unaffected by increased B light percentage in this study, which fits previous research on basil microgreens cultivated under LED supplying varying proportions of B light in the range of 33% to 67% (Lobiuc et al., 2017). However, total carotenoid concentration increased in beet microgreens when B light supplied from R:B LEDs was increased from 0 to 33%, but not in parsley or mustard microgreens (Samuolienė et al., 2017). As photosynthetic accessory pigments, carotenoids dissipate excess light energy by combining with singlet oxygen to prevent oxidative damage under high light (Jahns and Holzwarth, 2012). The possibility remains that in the present study that there was no impact of B light since microgreens were grown under the same *PPFD* of 300 $\mu mol \cdot m^{-2} \cdot s^{-1}$. This *PPFD* has been shown to be optimal for microgreen quality, including the detection of surface green coloration, and hence a lack of yellowing in microgreens (Jones-Baumgardt et al. 2019).

Nitrate is the main source of nitrogen that plant roots acquire from the soil, and is involved for example in the synthesis of proteins and chlorophyll in plants (Marschner, 1995). However, increased amount of dietary nitrate could lead to a negative impact on human health. In the present study, extractable nitrate levels were unaffected by LEDs generating varying proportions of B light percentage, regardless of species. This is consistent with a previous study on lettuce, where nitrate was found to be equivalent in 10% and 25% B light delivered from R:B LEDs at a *PPFD* of 200 $\mu mol \cdot m^{-2} \cdot s^{-1}$ (Zhang et al., 2018). The nitrate levels in *Brassicaceae* microgreens are lower than the maximum nitrate levels that are allowable for foodstuffs in some global markets (European Union, 2011). The lack of a B light effect on nitrate composition

fits with the observation that chlorophyll levels in all four *Brassicaceae* microgreens were not impacted by the proportion of B light in our study. This association is likely given the fact that nitrate availability in leaves affects the biosynthesis of chlorophyll and its precursors (Wen et al., 2019). Previous research indicated that ascorbate:nitrate ratios $>$ 1 occur in some greenhouse-grown vegetables, and this comparison is postulated to serve as a measure of the nutritional value of horticultural products (Pokluda, 2006). In this study, the total ascorbate: nitrate ratio ranged between 0.8 to 6.3 across all four microgreen species and their B light percentage treatments. In general, the average total ascorbate: nitrate ratios were 1.1 and 1.2 in red cabbage and kale, respectively, whereas these ratios were higher in arugula and mustard, with respective averages of 2.2 and 4.1. It is worth mentioning that the greatest total ascorbate: nitrate was apparent at 20% B light for all four microgreens assessed in this study. Thus, the *Brassicaceae* microgreens cultivated in the present study can be considered nutritious as per the Pokluda (2006) rationale.

3.4.2 Effect of B light in R:B LEDs on the concentrations of ascorbate, total phenolics and total anthocyanins in microgreens

With the exception of red cabbage, total and reduced ascorbate concentrations were elevated to a maximum in all microgreens with an increase in B light (i.e., 20% B light) relative to the lowest proportion of 5%. Our results mirror those of a previous study on lamb's lettuce (*Valerianella locusta* L.) where ascorbate levels were greatest at 10% to 20% B light supplied by LEDs in a greenhouse (Długosz-Grochowska et al., 2017). The biosynthesis of ascorbate is dependent on the presence of light and its related biochemical processes, such as the

photosynthetic electron transport pathway in leaf chloroplasts (Yabuta et al., 2007). It is known that increased proportions of B light generated by LEDs increase photosynthesis (Hogewoning et al., 2010; Matsuda et al., 2004; 2007). Thus, the accumulation of reduced and total ascorbate in the majority of microgreens could be due in part to the positive effect of B light on photosynthesis, although this physiological parameter was not assessed in this study. In our study, the possibility remains that an increase in the proportion of B light promotes increased gene expression and activity of enzymes operating in the ascorbate recycling pathway (e.g., ascorbate peroxidase), as these phenomena have been described for hydroponically-grown lettuce seedlings under LEDs supplying 75% B light relative to 25% R light (Zha et al., 2020). Interestingly, the aforementioned lettuce seedling study determined that an increase in the proportion of B light did not alter dehydroascorbate concentration. Similarly, dehydroascorbate concentration was unchanged by the proportion of B light for most of the *Brassicaceae* microgreens investigated in our study. The possibility remains that high dehydroascorbate levels in arugula microgreens at 15% to 20% B light relative to all other treatments could be due to an impairment in the ascorbate recycling pathway.

Increased proportions of B light promoted the accumulation of the total phenolics in kale and mustard. Similarly, total phenolic concentrations in two lettuce cultivars were higher under 47% B light generated by LEDs than plants cultivated under 0-13% and 0-35% B light for ‘Sunmang’ cultivar, and ‘Grand Rapid TBR’ cultivar, respectively (Son and Oh, 2013). However, total phenolics were not affected by B light in red cabbage and arugula microgreens in this study. These findings are consistent with the variable effect that R:B LED ratios have on the concentration of total phenolics in acyanic and cyanic basil microgreens (Lobiuc et al., 2017).

The increase in total phenolics with an elevation in B light percentage could be due to increased activity of phenylalanine ammonia-lyase, which is the initial step in the phenylpropanoid pathway leading to the production of flavonoids, including anthocyanins. In fact, high anthocyanin levels and phenylanaline ammonia lyase activity are evident in the leaves of red curly lettuce grown under R LEDs supplemented with B light relative to plants exposed to R or B light alone (Heo et al., 2012).

An increased percentage of B light in B:R LED arrays proportionally increased the total anthocyanin concentration in arugula, kale and red cabbage. Similarly, basil microgreens accumulated more anthocyanins under 50% and 66.7% B light than at 33.3% B light (Lobiuc et al., 2017). Overall, the accumulation of anthocyanins under increased B light percentage in the microgreens investigated in this study could be associated with more red coloration of microgreen cotyledons, a trait that was reported in a previous study (Ying et al., 2020). The increased levels of anthocyanins under high percentage of B light could be due to enhanced activities of key anthocyanin biosynthesis enzymes (e.g., anthocyanidin synthase) (Xu et al., 2014). B light promotes anthocyanin accumulation primarily through the action of cryptochromes, but it also requires active phytochrome proteins (Ahmad and Cashmore, 1997; Bouly et al., 2007; Kadomura-Ishikawa et al., 2013). It is tempting to speculate that cryptochrome-mediated upregulation of the anthocyanin biosynthesis pathway is involved in altering the levels of total anthocyanins in *Brassicaceae* microgreens.

3.5 CONCLUSION

Overall, for all *Brassicaceae* microgreens investigated in this study, the total chlorophyll, carotenoid and nitrate concentrations were not altered by the percentage of B light supplied by

LEDs during cultivation under controlled environment. By contrast, the concentrations of extractable antioxidants, including total phenolics, reduced and total ascorbate, and total anthocyanin were enhanced with increased proportions of B light, although the magnitude of the effect on these phytochemicals and/or the B light percentage(s) eliciting the maximal response varied across species. To this end apart from red cabbage, total ascorbate concentrations were smallest at 5% B light, and greatest at 20% B light, and slightly smaller at 30% B light. Increased supply of B light up to 30% was associated with the maximal accumulation of anthocyanins in arugula, kale and red cabbage, but not for mustard. It is worth mentioning that total anthocyanin concentrations were only 4 to 15% smaller at 20% than 30% B light. Thus as a compromise, 20% B light can be used for the cultivation of *Brassicaceae* microgreens containing high levels of ascorbate and anthocyanins.

CHAPTER FOUR

APPLYING BLUE LIGHT ALONE, OR IN COMBINATION WITH FAR-RED LIGHT, DURING NIGHTTIME INCREASES ELONGATION WITHOUT COMPROMISING YIELD AND QUALITY OF INDOOR-GROWN MICROGREENS[2]

ABSTRACT

To faciliate machine harvest for labour savings, the height of microgreen needs to reach approximately 5 cm. Recent studies indicate that monochromatic B light can promote stem elongation similarly to FR light. To examine whether B LED lighting treatments during night period (i.e., nighttime) can promote plant elongation without compromising yield and quality, mustard (*Brassica juncea*) and arugula (*Eruca sativa*) microgreens were grown under different LEDlighting regimes in a growth chamber. The 16-hour daytime lighting comprised 20% B and 80% R light ($B_{20}R_{80}$) and had a total *PPFD* of 300 $\mu mol \cdot m^{-2} \cdot s^{-1}$ at canopy level. During the 8-hour nighttime, the plants were exposed to the following treatments: (1) dark (D) as one control; (2) 4 hours of B light at 40 $\mu mol \cdot m^{-2} \cdot s^{-1}$ followed by 4 hours of darkness (40B-D); (3) 4 hours of darkness followed by 4 hours of B light at 40 $\mu mol \cdot m^{-2} \cdot s^{-1}$ (D-40B); (4) 8 hours of B light at 20 $\mu mol \cdot m^{-2} \cdot s^{-1}$ (20B); (5) 8 hours of B+FR light, and each of them at 20 $\mu mol \cdot m^{-2} \cdot s^{-1}$ (20B20FR); and (6) 8 hours of FR light at 20 $\mu mol \cdot m^{-2} \cdot s^{-1}$ (20FR) as another control. The plants were harvested after 11 days of treatments. Nighttime B treatments (40B-D, D-40B, and 20B), compared to D, increased the plant height by 34% and 18% for mustard and arugula, respectively, with no difference among the three B light treatments. The combination of B and FR light (20B20FR),

[2] *Article citation*: Ying, Q., Y. Kong, and Y. Zheng. 2020. Applying blue light alone, or in combination with far-red light, during nighttime increases elongation without compromising yield and quality of indoor-grown microgreens. HortScience 55:876-881.

compared to B light alone, further increased plant height by 6% and 15% for mustard and arugula, respectively, and showed a similar promotion effect as 20FR. Plant height did not meet the machine harvest requirement for both species under D, but did so for mustard under nighttime B treatments and for arugula under 20B20FR. There was no difference in biomass among all the treatments except that 20B, compared to D, increased fresh weight of arugula by 12%, showing a similar promotion effect as 20FR. Despite a greater promotion effect on elongation than B light alone, 20FR reduced leafy index compared to D. However, B light alone or 20B20FR treatment increased leaf thickness compared to D, and increased chlorophyll content index, leafy index, dry matter content, and leaf thickness to varying degree with species, compared to 20FR. Overall, nighttime B light alone, or its combination with FR, promoted microgreen elongation without compromising yield and quality.

4.1 INTRODUCTION

Microgreens are edible seedlings harvested when their cotyledons fully expanded, with or without the appearance of true leaves depending on species (Treadwell et al., 2016). Within 80 to 100 plant species that are currently cultivated as microgreens, the most common species are from the *Brassicaceae* family, including mustard and arugula (Björkman et al., 2011). As specialty vegetables, microgreens have various colors and flavors, tender textures, and high nutritional contents (Xiao et al., 2012; Kyriacou et al., 2016). The growth periods of microgreens are relatively short, as they are normally harvested within 7–20 d after sowing (Treadwell et al., 2016).

Microgreens have been increasingly produced in controlled-environment facilities with electric lighting as sole light source, LEDs. LED lighting has made it possible to choose the

specific light intensity and spectral composition (Morrow, 2008), which can be used to regulate growth and development according to plant species (Bergstrand, 2017). LED light with a combination of R light (600 to 700 nm) and B light (400 to 500 nm) has been popularly used for horticultural production in recent years (Davis and Burns, 2016). For indoor production of microgreens, RB-LED lighting with 15–20% B light and 300–400 $\mu mol \cdot m^{-2} \cdot s^{-1}$ *PPFD* under 16-h photoperiod appears to be an optimal lighting regime in terms of crop yield and appearance quality (Ying et al., 2020; Jones-Baumgardt et al., 2019). However, under the optimized lighting environment, microgreens are too short to be harvested by machine. Commercial productions of microgreens have been increasingly switching from hand- to machine-harvesting to reduce labour cost. Microgreens with hypocotyls < 5 cm are not feasible to be harvested by machine (personal communications with some Canadian microgreen growers). Also, most microgreen species are harvested with a minimum height of around 5 cm according to Kyriacou et al. (2016).

Although plant elongation can be promoted by creating a shade environment during daytime, there is normally a trade-off between stem elongation and plant yield under shade (Tang and Liesche, 2017). Also, our previous studies on microgreens indicated that adjusting spectral quality of daytime lighting, like adding small amount of FR or G light to RB-LED lighting did not change plant elongation or even had negative effect on appearance quality of four microgreen species (Ying et al., 2020a). Substituting G or FR light for B light in RB-LED lighting, or adding FR to RB-LED lighting reduced leaf greenness or redness in lettuce and kale, or reduced relative specific chlorophyll content in lettuce and basil seedlings despite the promoted elongation responses of leaves or hypocotyl (Meng et al., 2019; Meng and Runkle,

2019). Although lowering B light percentage in daytime RB-LED lighting sometimes can also promote plant height, the effects vary with species, and also can compromise other growth metrics, such as reducing leaf thickness, relative dry weight, shoot fresh weight or leaf red color (Meng and Runkle, 2019; Nanya et al.,2012; Wollaeger and Runkle, 2015; Ying et al., 2020b), and health-promoting phytochemical contents (See Chapter 3).

To promote stem elongation without compromising other growth metrics, it may be feasible to extend the daily lighting period during night (e.g., longer photoperiod). Longer photoperiod is normally associated with increased daily light integral (DLI), which has been found to induce taller plants and greater biomass in some plant species, like vinca (*Vinca minor* L.) and zinnia (*Zinnia elegans* L.) (Faust, 2002), and pea shoots (*Pisum sativum* L.) (Kong et al., 2018b). For indoor microgreen production, even 24-h photoperiod (i.e., 0-h dark) did not cause obvious negative effect on plant quality and induced a greater promotion effect on stem elongation compared to 16-h photoperiod (Kong et al., 2019b). The nighttime lighting could be particularly useful in some regions, like Ontario, where the electricity price is much lower during nighttime compared to the daylight hours. Also, the crop cycles can be potentially shortened by applying nighttime lighting, which may speed up the cash flow to some degree. However, the optimal spectral quality and lighting regimens at night for indoor-grown microgreens are unknown.

Promotion of stem elongation has been widely documented under environments containing high-level FR (Demotes-Mainard et al., 2016). The elongation response to FR light is a typical shade-avoidance response mediated by phytochrome, a photoreceptor that is mainly activated by R but deactivated by FR (Sager et al., 1988). End-of-day FR treatment has been

reported to enhance stem elongation in tomato (Kubota et al., 2012); chrysanthemum (*Chrysanthemum morifolium* L.) (Lund et al., 2007); and poinsettia (*Euphorbia pulcherrima* L.) (Islam et al., 2014). Nighttime FR light was also extremely useful in promoting shoot elongation during the early stage for the seedlings of Japanese pear (*Pyrus pyrifolia* (Burm. f.) Nakai) (Ito et al., 2014). However, plants grown under high-level FR light environments might undergo some undesirable responses, such as decreased chlorophyll content and leaf thickness (Demotes-Mainard et al., 2016). Furthermore, FR LEDs have not been popularly available for horticultural production until recent years, due to the higher price in the past (Kubota et al., 2012), and lower photosynthetic photon efficiency relative to R and B light (Nelson and Bugbee, 2014).

Apart from FR, monochromatic B LED lighting at modest intensities (ranging from 40 to 250 $\mu mol \cdot m^{-2} \cdot s^{-1}$) has also been reported to promote stem elongation in some plant species such as 'Cuty' cherry tomato (Kim et al., 2014), cucumber (Hernández and Kubota, 2016), sesame (*Sesamum indicum* L.) (Hata et al., 2013), and sunflower (*Helianthus annuus* L.) (Schwend et al., 2015). Recently, based on a series of 24-h LED lighting experiments on bedding plants, microgreens, and *Arabidopsis* mutants, our lab has concluded that the promotion effect of monochromatic B light is related to low phytochrome activity, which may modify the activity of B receptors (e.g., reduced cryptochrome activity and increased phototropin activity) (Kong et al., 2018a; Kong et al., 2019a; Kong and Zheng, 2020). Also, for arugula (*Eruca sativa*) and mustard (*Brassica juncea*) microgreens under 24-h lighting, the promoted elongation by monochromatic B increased with *PPFD* decreasing from 650 to 20 $\mu mol \cdot m^{-2} \cdot s^{-1}$ (Johnson et al., 2020). However, the stem elongation promoted by 24-h lighting of modest-intensity B light is shade-avoidance response in plants, since reduced cotyledons size, and side branch number,

and increased biomass partitioning to stem have been observed at the same time (Kong et al., 2019a; 2019b). It is unknown whether whole-night lighting (i.e., total 24 h photoperiod) with a low intensity (e.g., 20–40 $\mu mol \cdot m^{-2} \cdot s^{-1}$) of monochromatic B can promote elongation without compromising yield and quality, for indoor microgreen production with16-h daytime RB-LED lighting.

Although both B and FR light can promote stem elongation, there is not enough information comparing the promotion effect among B, FR and B+FR light. For microgreens (i.e., arugula, cabbage, and kale) under 24-h lighting, B plus low-level FR light slightly promoted plant elongation compared to B light, but the promotion effect was far less than that of B relative to R light (Kong et al., 2019c). It appears that B has a similar or greater promotion effect on microgreen elongation compared to FR, since more than one photoreceptor system (i.e. phytochromes) is involved in the promotion effect of B rather than FR light (Kong and Zheng, 2020). However, in the aforementioned microgreen study, the intensity of FR light was only 10% of the B light intensity. A recent study indicated that although FR light alone minimally affect plant photosynthesis, it is equally efficient at driving canopy photosynthesis when it is co-acting with photosynthetically active radiation (*PAR*) (Emerson et al., 1957; Zhen and Bugbee, 2020). Possibly, the combination of B and FR light at the same levels could increase plant biomass. Therefore, the promotion effects on plant elongation as well as biomass need to be compared among B, FR, and B+FR light as whole-night lighting (i.e., total 24-h photoperiod) for indoor microgreen production under 16-h daytime RB-LED lighting.

For microgreens, when photoperiod is reduced from 24 h to 16 h, the hypocotyl elongation was still promoted by monochromatic B light, and met the height requirement of

machine harvest (i.e., ≥ 5 cm), although the promotion effect was reduced to some extent (Kong et al., 2019b). As for nighttime B light treatments, it remains unclear when total DLI is the same, whether partial vs. whole nighttime lighting (e.g., 20- vs. 24-h photoperiod) would affect the promotion effect on elongation. Also, for partial nighttime lighting (e.g., 20-h photoperiod), when to apply B light (i.e., early or late nighttime) would have a greater promotion effect is unknown. It has been found that B light reduced promotion effect and gradually switched to inhibition effect on plant elongation relative to R light, when phytochrome photostationary state (PPS), an indicator of phytochrome activity, increased from 0.50 to 0.69 (Kong et al., 2018a; Kong et al.,2019c). Apparently, the activity of cryptochrome, B light photoreceptor, was increased by activated phytochrome, possibly due to a crosstalk between the two photoreceptors systems (Liu et al., 2016). A previous study on cucumber (*Cucumis sativus* L.) seedlings indicated that phytochrome still remained activated after lights off and might last for several hours in the dark, although the phytochrome activity gradually decreased over time (Gaba and Black, 1979). Consequently, the effect of nighttime B light on plant elongation may differ between early and later hours during the nighttime due to the difference in phytochrome activity. This speculation needs to be confirmed for indoor microgreen production under 16-h daytime RB-LED lighting.

Although many previous studies have reported the promotion effect of B light as sole light source on plant elongation, little information is available on the application of B light as supplemental lighting source to indoor microgreen production. Therefore, the objective of this study was to (1) evaluate the effect of nighttime B light, or its combination with FR light treatments, relative to dark and FR light, on hypocotyl elongation, as well as crop yield and

appearance quality; and (2) under equal DLI, to determine the optimum nighttime lighting strategy (e.g., initiation time and duration) using B light for indoor microgreen production with 16-h RB-LED lighting during daytime.

4.2 MATERIALS AND METHODS

4.2.1 Plant materials and growing conditions

This experiment was performed in a walk-in growth chamber (7.3 m × 4.0 m × 2.5 m), which was divided into six compartments by opaque curtains, from October to November of 2018. Seeds of arugula (*Eruca sativa*; 'unknown name', Suba Seeds Company S.P.A., Longiano, FC, Italy) and mustard *(Brassica juncea*; 'Mizuna', Johnny's Selected Seeds, Winslow, ME, USA) were sown in fiber trays (48.5 × 23 × 3.5 cm), that were filled with substrate consisting of 30% compost, 30% peat, 30% coir, and 10% perlite (v/v). The seeding rate was 36 $g \cdot m^{-2}$ for both arugula and mustard, and each tray had one species. After seeding, the sown trays were placed on the bench in each compartment for lighting treatments. The substrate was prepared by Greenbelt Microgreens Ltd. (Lynden, ON, Canada), and had a pH of 6.9 and electrical conductivity (EC) of 1.9 $dS \cdot m^{-1}$. The nutrient content ($mg \cdot kg^{-1}$) in the substrate was measured by a commercial analytical lab (A&L Canada Laboratories Inc., London, ON, Canada) using test methods for composting and compost, and the result was as follows: N 9400, P 329, K 1410, S 220, Mg 295, Ca 1390, Zn 9.4, Mn 10, Fe 47, Cu 0.6, Na 218, and B 0.7. All trays were top irrigated at least daily using well water (pH = 7.5; EC = 0.8 $dS \cdot m^{-1}$) until visible drainage. The temperature was set at 20 °C during daytime (10:00 AM to 02:00 AM) and 17 °C during nighttime, and the relative humidity (RH) was set at 70%. Temperature and RH were controlled by an environmental control system (Titan, Argus Controls Systems Ltd., Surrey, BC, Canada).

4.2.2 Experimental design and treatments

The experiment was a randomized complete block design with six treatments and three consecutive experimental replicates. The six treatments only differed during the 8-h nighttime, but had the same 16-h daytime lighting, which was provided by RB-LED with a photon flux ratio of 20% B and 80% R ($B_{20}R_{80}$) at a *PPFD* of 300 µmol·m^{-2}·s^{-1} from 10:00 AM to 02:00 AM. The six nighttime treatments were: (1) D, dark for 8 h as one control; (2) 40B-D, 40 µmol·m^{-2}·s^{-1} B light for the first 4 h and dark for last 4 h; (3) D-40B, dark for the first 4 h and 40 µmol·m^{-2}·s^{-1} B light or last 4 h; (4) 20B, 20 µmol·m^{-2}·s^{-1} B light for 8 h; (5) 20B20FR: 20 µmol·m^{-2}·s^{-1} B light and 20 µmol·m^{-2}·s^{-1} FR light together for 8 h ; (6) 20FR: 20 µmol·m^{-2}·s^{-1} FR light for 8 h as another control. Both day and nighttime light treatments were provided solely by LEDs (LX602C, Heliospectra AB, Gothenburg, Sweden). For each replicate, the six treatments were randomly allocated to the six compartments in the growth chamber, and there were two trays of microgreens (one species per tray) under each treatment. The trial was replicated three times over time, and the treatment locations were randomized within the growth chamber for each replication.

In each compartment, two LED lamps were hung 56.5 cm above the top of the substrate (measured from the bottom of the lamps) and separated by 36 cm (on-center). The individual B and R channels were set to achieve an average *PPFD* of 300 µmol·m^{-2}·s^{-1} during the 16-h daytime. Additionally, the B and FR channels were set up to provide the various nighttime spectrum treatments, all of which had an average photon flux density (*PFD*) of 20 µmol·m^{-2}·s^{-1} or 40 µmol·m^{-2}·s^{-1}. The actual lighting conditions are summarized in Table 4.1. The PPS values for the daytime lighting and the different nighttime lighting were calculated using the protocol

(Mah et al., 2019) developed by our lab according to Sager et al. (1988). The daily light integral (DLI) and daily photon integral (DPI) were calculated using the total photons from 400–700 nm and 400–800 nm, respectively. Photon flux density and spectra of the LED arrays were controlled by using a software (System Assistant Version 1.3.0.; Heliospectra, AB). Photon flux density and spectrum were measured at 16 spots (a 4 × 4 grid) within a square treatment area of 0.25 m^2, centered between the LED fixtures, using a radiometrically-calibrated spectrometer (Flame-S, Ocean Optics, Inc., Dunedin, FL) with a 25 µm slit, coupled to a 1.89 m × 400 µm fiber optic patch chord with a CC-3 cosine corrector. Light intensity and spectra were also checked at the end of each replicate.

Table 4.1. Photosynthetic photon flux density (PPFD) from blue (B; 400–500 nm), red (R; 600–700 nm) light, combined and phytochrome photostationary state (PPS) during daytime, and average PFD from B, far-red (700–800 nm) light, and PPS during nighttime, and the daily photon integral (DPI) under six light spectral treatments.

Night treatment	Day time PPFD ($\mu mol \cdot m^{-2} \cdot s^{-1}$)[z] and PPS				Nighttime PPFD ($\mu mol \cdot m^{-2} \cdot s^{-1}$) and PPS			DPI[y] ($mol \cdot m^{-2} \cdot d^{-1}$)	DLI ($mol \cdot m^{-2} \cdot d^{-1}$)
	Blue	Red	*PAR*[y]	PPS	Blue	Far-red	PPS		
D[x]	60 ± 0.5	239 ± 2.1	299 ± 2.6	0.88 ± 0.0	-	-	-	17.3	17.3

40B-D	60 ± 0.5	239 ± 1.3	299 ± 1.4	0.88 ± 0.0	40 ± 0.3	-	0.53 ± 0.0	17.9	17.9
D-40B	60 ± 0.3	239 ± 1.2	300 ± 1.3	0.88 ± 0.0	41 ± 0.2	-	0.53 ± 0.0	17.9	17.9
20B	60 ± 0.8	237 ± 0.4	298 ± 1.3	0.88 ± 0.0	20 ± 0.4	-	0.52 ± 0.0	17.9	17.9
20B20FR	61 ± 0.7	238 ± 1.1	300 ± 2.2	0.88 ± 0.0	20 ± 0.5	20.3 ± 0.5	0.23 ± 0.0	18.5	17.9
20FR	61 ± 0.9	239 ± 2.5	300 ± 1.9	0.88 ± 0.0	-	19.7 ± 0.4	0.18 ± 0.0	17.9	17.3

[z]Data are means ± SE of three experimental replicates.

[y]PPS: phytochrome photostationary state, which is the estimated phytochrome photoequilibrium according to the method by Sager et al. (1988). *PAR*: photosynthetically active radiation ranging from 400 to 700 nm. DPI: daily photon integral ($mol \cdot m^{-2} \cdot d^{-1}$), ranging from 400 to 800 nm.

[x]D: dark for 8 h; 40B-D: 40 $\mu mol \cdot m^{-2} \cdot s^{-1}$ blue (B) light for the first 4 h and dark for last 4 h; D-40B: dark for the first 4 h and 40 $\mu mol \cdot m^{-2} \cdot s^{-1}$ B light for the last 4 h; 20B: 20 $\mu mol \cdot m^{-2} \cdot s^{-1}$ B light for 8 h; 20B20FR: 20 $\mu mol \cdot m^{-2} \cdot s^{-1}$ B light and 20 $\mu mol \cdot m^{-2} \cdot s^{-1}$ far-red (FR) light together for 8 h; 20FR: 20 $\mu mol \cdot m^{-2} \cdot s^{-1}$ FR light for 8 h. The six nighttime treatments had the same 16-h daytime lighting, which was provided by a combination of 20% B and 80% red LEDs at a *PPFD* of 300 $\mu mol \cdot m^{-2} \cdot s^{-1}$ from 10:00 AM to 02:00AM.

4.2.3 Data collection and analyses

Both arugula and mustard microgreens were harvested 11 d after seeding. Plants were harvested with two fully expanded cotyledons and visual observation of the first emerged true leaves. For each replicate and each species, three subsamples (with a substrate surface area of 76.4 cm^2 each including plants and the substrate) of microgreens were randomly sampled from each tray using a cylindrical core sampler. All the microgreens in each subsample were then cut at substrate level and measured for FW. These plants were then dried at 65 °C until a constant weight was attained to determine DW. Twenty-five plants from each genotype were randomly selected from the remainder of the microgreens in each tray to evaluate plant height. Another 15 plants (5 plants × 3 sets) were selected randomly from the plants left in each tray and separated into cotyledons and hypocotyls for quality evaluations. After measuring FW of cotyledons and stems separately, they were imaged using a flat-bed digital scanner (Canoscan LiDE 25, Canon Inc., Japan). The images were saved in JPG format with 600 dpi resolution, and later they were processed using ImageJ 1.42 software (National Institute of Health, USA) to determine cotyledon area. After scanning, the cotyledons and hypocotyls were dried separately at 65 ℃ till constant weight and DW were recorded. The leafy index, dry matter content and specific leaf area (SLA) were determined for the 3 sets of 5 plants from each replicate and each treatment and were calculated as:

$$\text{Leafy index } (g \cdot g^{-1}\ DW) = DW \text{ of cotyledons} / DW \text{ of hypocotyls} \quad [1]$$

$$\text{Dry matter content } (\%) = DW/FW \times 100 \quad [2]$$

$$SLA\ (cm^2 \cdot g^{-1}) = \text{Cotyledon area} / DW \text{ of cotyledons} \quad [3]$$

Another 15 plants from each tray were selected randomly to measure the chlorophyll content index (CCI) of the cotyledons using a chlorophyll meter (SPAD 502; Spectrum Technologies, Inc., Aurora, IL).

Data were subjected to analysis of variance (ANOVA) using the SPSS software (Version 25.0, IBM, New York) followed by multiple comparisons using Tukey's honestly significant difference (HSD) test at $P \leq 0.05$. Data were presented as mean ± SE, n=3.

4.3 RESULTS

Compared to the D treatment, all nighttime B light treatments increased plant height, with similar trends in both microgreen species (Fig. 4.1). The average plant heights under the nighttime B light treatments (i.e., 40B-D, D-40B, and 20B) were 6.4 cm and 4.2 cm for mustard and arugula, respectively. The three B light treatments promoted plant height in a similar manner in both species, and increased plant height by 34% for mustard (Fig. 4.1A) and 18% for arugula (Fig. 4.1B), respectively, compared to the D treatment. The combination of B and FR light treatment (20B20FR), compared to B light alone, further increased plant height by 6% and 15% for mustard and arugula, respectively, and showed a similar promotion effect as 20FR. The plant height under 20B20FR treatment was 6.8 cm and 5.1 cm for mustard and arugula, respectively.

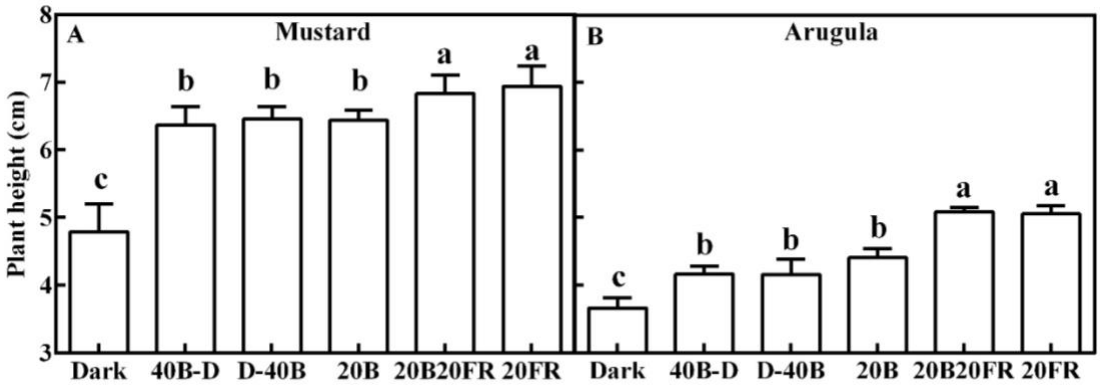


Figure 4.1. Effect of nighttime light treatments on height of mustard (A) and arugula (B) microgreens. Data are mean ± SE of three separate experimental replicates. Bars sharing the same letter are not significantly different by Tukey's honestly significant difference test at $P \leq 0.05$. D: dark for 8 h; 40B-D: 40 $\mu mol \cdot m^{-2} \cdot s^{-1}$ blue light for the first 4 h and dark for last 4 h; D-40B: dark for the first 4 h and 40 $\mu mol \cdot m^{-2} \cdot s^{-1}$ blue light for the last 4 h; 20B: 20 $\mu mol \cdot m^{-2} \cdot s^{-1}$ blue light for 8 h; 20B20FR: 20 $\mu mol \cdot m^{-2} \cdot s^{-1}$ blue light and 20 $\mu mol \cdot m^{-2} \cdot s^{-1}$ far-red light together for 8 h; 20FR: 20 $\mu mol \cdot m^{-2} \cdot s^{-1}$ far-red light for 8 h. The six nighttime treatments had the same 16-h daytime lighting, which was provided by a combination of 20% blue and 80% red LEDs at a *PPFD* of 300 $\mu mol \cdot m^{-2} \cdot s^{-1}$ from 10:00 AM to 02:00AM.

The FW of mustard was not affected by any of the nighttime treatments (Fig. 4.2A). The FW of arugula was 12% higher under both 20B and 20FR, compared to D, and 20B showing a similar promotion effect as 20FR (Fig. 4.2B). There were no treatment effects on DW in mustard or arugula (Fig. 4.2C-D).

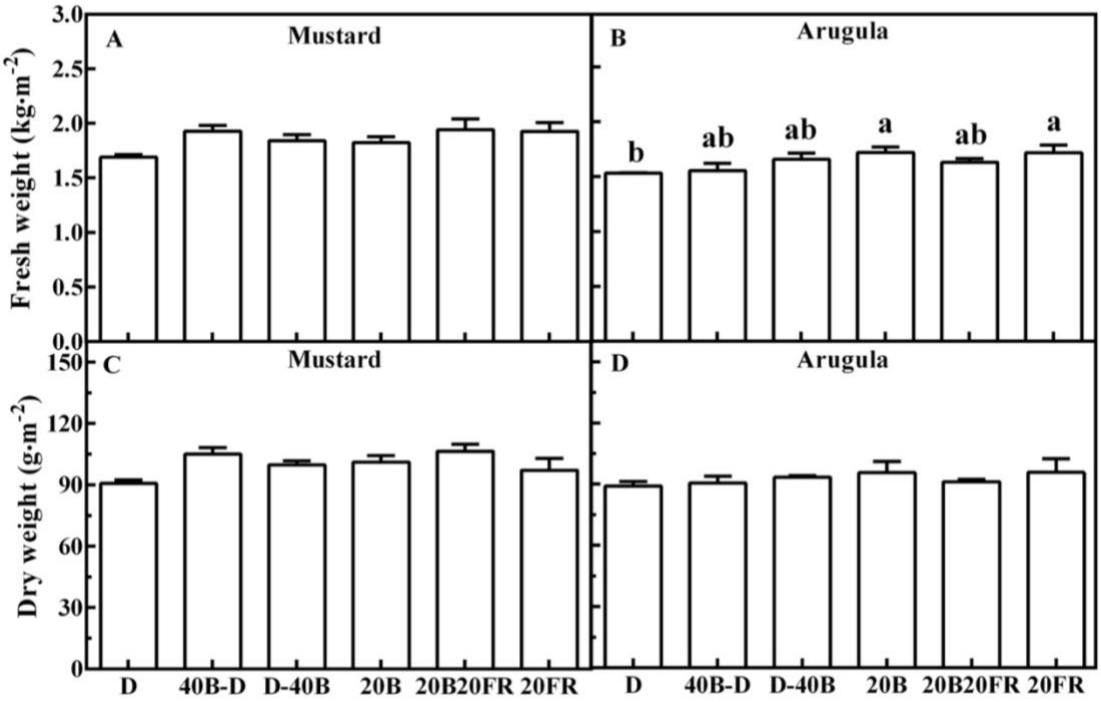


Figure 4.2. Effect of nighttime light treatments on fresh weight (A–B) and dry weight (C–D) of mustard and arugula microgreens. Data are mean ± SE of three separate experimental replicates. Bars bearing the same letter are not significantly different by Tukey's honestly significant difference test at $P \leq 0.05$. D: dark for 8 h; 40B-D: 40 $\mu mol \cdot m^{-2} \cdot s^{-1}$ blue light for the first 4 h and dark for last 4 h; D-40B: dark for the first 4 h and 40 $\mu mol \cdot m^{-2} \cdot s^{-1}$ blue light for the last 4 h; 20B: 20 $\mu mol \cdot m^{-2} \cdot s^{-1}$ blue light for 8 h; 20B20FR: 20 $\mu mol \cdot m^{-2} \cdot s^{-1}$ blue light and 20 $\mu mol \cdot m^{-2} \cdot s^{-1}$ far-red light together for 8 h; 20FR: 20 $\mu mol \cdot m^{-2} \cdot s^{-1}$ far-red light for 8 h. The six nighttime treatments had the same 16-h daytime lighting, which was provided by a combination of 20% blue and 80% red LEDs at a *PPFD* of 300 $\mu mol \cdot m^{-2} \cdot s^{-1}$ from 10:00 AM to 02:00AM.

Cotyledon area of arugula was increased by 21% under D-40B as compared to the D treatment, while other treatments had no effect on this trait in either species (Table 4.2). Leafy

indices were higher in mustard under D, D-40B and 20B20FR than 20FR, and in arugula under 20B and 20B20FR than D. The dry matter content was lower under 20FR compared to all other treatments for mustard, but was not different among all the treatments for arugula. The CCI of the cotyledons was increased by 12%, 13% and 10% under D-40B, 20B and 20B20FR, respectively, for mustard, and by 9% under 20B20FR for arugula, compared to 20FR. The SLA of arugula decreased by 17% under D-40B, 20B and 20B20FR compared to D, and by 15% under 20B20FR compared to 20FR, but this trait showed no difference among all the treatments for mustard.

Table 4.2. Effect of nighttime light treatments on cotyledon area, leafy index, dry matter content, chlorophyll content index (CCI), and specific leaf area (SLA) of arugula and mustard microgreens.

Night treatment	Cotyledon area (cm^2/plant)	Leafy index[y] ($g \cdot g^{-1}$)	Dry matter content (%)	CCI	SLA ($cm^2 \cdot g^{-1}$)
			Mustard		
D[x]	2.3 ± 0.2 [z]	2.6 ± 0.5 a	5.4 ± 0.0 ab	32.8 ± 1.3 ab	78.6 ± 0.2
40B-D	2.5 ± 0.1	2.3 ± 0.5 ab	5.5 ± 0.1 a	32.6 ± 0.6 ab	74.3 ± 7.5
D-40B	2.3 ± 0.1	2.3 ± 0.6 a	5.4 ± 0.1 a	34.5 ± 0.6 a	77.5 ± 1.7
20B	2.3 ± 0.2	2.2 ± 0.6 ab	5.6 ± 0.1 a	34.8 ± 0.6 a	74.4 ± 1.6
20B20FR	2.5 ± 0.2	2.4 ± 0.6 a	5.5 ± 0.1 a	33.9 ± 0.6 a	68.8 ± 4.7
20FR	2.3 ± 0.2	1.8 ± 0.2 b	5.1 ± 0.2 b	30.9 ± 0.3 b	77.6 ± 3.1
			Arugula		

D	2.6 ± 0.2 b	3.0 ± 0.3 a	6.2 ± 0.5	33.6 ± 1.6 ab	70.5 ± 0.9 a
40B-D	2.9 ± 1.9 ab	2.7 ± 0.3 ab	5.9 ± 0.3	33.4 ± 0.8 ab	65.2 ± 2.8 ab
D-40B	3.1 ± 0.3 a	2.8 ± 0.4 ab	5.7 ± 0.2	34.1 ± 1.1 ab	61.7 ± 3.9 bc
20B	2.8 ± 0.2 ab	2.2 ± 0.1 bc	5.7 ± 0.1	35.0 ± 0.0 ab	59.3 ± 3.5 bc
20B20FR	2.9 ± 0.2 ab	2.2 ± 0.3 bc	5.8 ± 0.1	35.3 ± 0.3 a	55.4 ± 2.6 c
20FR	2.8 ± 0.2 ab	1.9 ± 0.0 c	5.7 ± 0.2	32.5 ± 0.3 b	65.3 ± 7.5 ab

[z]Data are means ±SE of three experimental replicates. Letters are only shown when there is significant treatment effect at $P \leq 0.05$. Means sharing the same letter are not significantly different by Tukey's honestly significant difference test at $P \leq 0.05$.

[y]Leafy index ($g \cdot g^{-1}$) = DW of cotyledons/ DW of hypocotyls

[x]D: dark for 8 h; 40B-D: 40 $\mu mol \cdot m^{-2} \cdot s^{-1}$ blue (B) light for the first 4 h and dark for last 4 h; D-40B: dark for the first 4 h and 40 $\mu mol \cdot m^{-2} \cdot s^{-1}$ B light for the last 4 h; 20B: 20 $\mu mol \cdot m^{-2} \cdot s^{-1}$ B light for 8 h; 20B20FR: 20 $\mu mol \cdot m^{-2} \cdot s^{-1}$ B light and 20 $\mu mol \cdot m^{-2} \cdot s^{-1}$ far-red (FR) light together for 8 h; 20FR: 20 $\mu mol \cdot m^{-2} \cdot s^{-1}$ FR light for 8 h. The six nighttime treatments had the same 16-h daytime lighting, which was provided by a combination of 20% blue and 80% red LEDs at a *PPFD* of 300 $\mu mol \cdot m^{-2} \cdot s^{-1}$ from 10:00 AM to 02:00AM.

4.4 DISCUSSION

4.4.1 Nighttime B light increases elongation growth, with a promotion effect comparable to far-red light, for indoor microgreen production

In contrast to the D treatment (i.e., no lighting at night), nighttime B light treatments (i.e., 40B-D, D-40B, and 20B) promoted elongation by 34% and 18% for mustard and arugula,

respectively, in the present study. Apparently, the extended lighting time contributed to the elongation growth. Normally, the stem elongation increased by extended lighting time could be caused by increased photo-assimilation for growth, and/or the production of light-dependent elongation-promoted hormone (Bergstrand, 2017; Warrington and Norton, 1991; Yamaguchi, 2008). In the present study, there was a less possibility of increased assimilation supply by nighttime lighting, which was also supported by similar dry biomass accumulation in these B treatments relative to the dark treatment. During the relatively short growth period (from seeding to cotyledon fully unfolding), it seems that microgreens mainly experience a transition from heterotrophic to autotrophic growth, and photosynthesis contributes little to elongation growth (Jones-Baumgardt et al., 2019). In a previous study on petunia, high levels of bioactive gibberellins were found under B light, which was related to B light-enhanced elongation growth (Fukuda et al., 2016). Therefore, gibberellin production could have been increased by the extended lighting with monochromatic B at night for the tested microgreen species in the present study, although it needs further confirmation.

The effect of nighttime B light exposure on the promotion of height elongation growth was comparable to FR in the present study. Under nighttime B light treatment, the microgreen plants were only 8% and 17% shorter compared to grown under FR light for mustard and arugula, respectively. Also, the plant height of mustard under nighttime B light treatments reached the > 5 cm requirement for machine harvest, suggesting that nighttime B light can be potentially used to promote plant elongation for microgreen production. The recent studies in our lab found that the promoted elongation growth by B light is related to low phytochrome activity, indicated by lower PPS value (Kong et al., 2018a; 2019c). The threshold value of PPS to

induce activated phytochrome responses is controversial, although the common belief is that PPS > 0.6 could generally induce these responses (Stutte, 2009). In the present study, the PPS values were lower than 0.6, indicating potentially deactivated phytochrome response, under all nighttime B and FR light treatments. However, B light treatments had a relatively higher PPS value than FR (0.52 vs. 0.18), which may explain the slightly reduced promotion effect on elongation.

4.4.2 Nighttime B vs. far-red light is better for indoor microgreen production in terms of yield and quality

In the present study, 20 $\mu mol \cdot m^{-2} \cdot s^{-1}$ B light applied throughout the nighttime for 8 h increased microgreen FW by 12% in arugula as compared to the D treatment, showing a biomass effect similar to that promoted by FR. Similarly, FW of Chinese kale (*Brassica alboglabra* 'Bailey') was increased by 41% when 50 or 100 $\mu mol \cdot m^{-2} \cdot s^{-1}$ of B light was supplemented for 12 h in a greenhouse (Li et al. 2019). The increased FW of arugula under 20B would potentially promote yield, and benefit production, since microgreens are sold on a FW basis. Furthermore, B light as opposed to FR has ever been more popularly available for a long time due to lower price of the LED lighting fixture, and higher photosynthetic photon efficiency (Kubota et al.,2012; Nelson and Bugbee, 2014). However, recent years the price of FR light LED has also been decreasing rapidly, so the comparison of economic effects between the two LED lighting needs further studies.

Extended lighting using B light alone at night also increased microgreen quality compared to dark or FR in many cases. For example, cotyledon area and thickness (i.e., reciprocal of SLA)

were increased for arugula under B light treatments compared to dark; dry matter content and CCI were increased for mustard, and leafy index was enhanced for both species under B light alone compared to FR. These quality improvements under nighttime B light treatments would potentially benefit microgreen production, since larger and greener cotyledons are more appealing in appearance to most consumers. Also, higher dry matter content and thicker leaves can potentially increase microgreen shelf life, since microgreens are highly perishable products (Di Gioia et al., 2015). Taking into account the negative effects of FR on cotyledon area, leaf thickness and stem diameter, monochromatic B light appears to be a better nighttime spectrum treatment for increasing plant height of indoor-grown microgreens.

4.4.3 Strategies of using nighttime B light for indoor microgreen production

In the present study, the shorter time (i.e., 4 h) of B light treatments (i.e., D-40B and 40B-D) at night did not affect microgreen stem elongation, yield or quality, compared to longer (i.e., 8 h) periods of B light exposure (i.e. 20B). This differed from a recent study in our lab, where shortening the daily lighting time from 24 h to 16 h reduced the promotion effects of monochromatic B light on stem elongation, cotyledon size, and plant color in microgreens to some degree (Kong et al., 2019b). Also, under natural light conditions in the greenhouse, shortened photoperiod inhibited plant elongation in some species through reduced DLI (Bergstrand, 2017). In the present study, although the duration of nighttime B light treatment was shortened from 8 h to 4 h, the total DLI did not change because a two-fold light intensity (40 vs. 20 $\mu mol \cdot m^{-2} \cdot s^{-1}$) was used for 4-h B lighting. However, in the previous studies, the

shortened photoperiods were also accompanied with lower DLIs. This may partly explain the inconsistency between the present study and other ones.

For partial nighttime (i.e., 4-h rather than 8-h) lighting, B light treatments at earlier vs. later night hours (i.e., 40B-D vs. D-40B) had similar effects on microgreen elongation, yield and quality in the present study. The sole-source RB-LEDs used for daytime lighting in the present study should induce a relatively high phytochrome activity (PPS = 0.88; Table 4.1) immediately after the light was off. However, monochromatic B light may need some time to reduce the PPS. For white mustard (*Sinapsis alba*) seedlings, around 30 min was needed for monochromatic B light to reduce the PPS value to initiate inactive phytochrome responses (Jabben et al., 1982). Possibly, at the beginning (e.g., 30 min) of the 4-h nighttime B light treatments, plant elongation was inhibited to a larger degree under the 40B-D compared to D-40B treatment due to gradually decreased phytochrome activity at night. However, this greater inhibition at the beginning under 40B-D vs. D-40B treatment might be eliminated by the elongation promoted by later 4-h dark. The underlined mechanism still needs to be further studied. Nevertheless, the flexible time window to apply B light during nighttime would provide an opportunity to lower lighting cost, since the electricity price is much lower during off-peak period in some regions.

The combination of B and FR light further promoted plant elongation compared to B alone. and showed a promotion effect similarly to FR light, but exerted no additive effect on yield or other quality metrics. Similarly, after adding low-level (10% total *PPFD*) of FR light to B light, the plant heights were increased slightly in arugula and mustard microgreens under 24-h sole-source LED lighting compared to B (Kong et al., 2019c). The increases in plant height under

B + FR light, compared to B light, may have resulted from further reduced phytochrome activity, indicated by a lower PPS value (i.e., 0.23 vs. 0.52). However, the sensitivity of elongation response to the decreased phytochrome activity seems to vary among species. In the present study, the plants grown under monochromatic B light alone treatments met the 5 cm height requirement for machine harvest for mustard, but not for arugula. For arugula, only B together with FR light could promote elongation sufficiently to meet the height requirement. It appears that for indoor microgreen production, B alone, or together with FR light can be used as nighttime lighting for promoting elongation, depending on plant species sensitivity to the decreased phytochrome activity.

4.5 CONCLUSION

In summary, for indoor production of mustard and arugula microgreens under 16-h daytime RB-LED lighting, treatment 40B-D, D-40B, and 20B increased plant height for both species compared to D, and had no differences among the three B light treatments. Treatment 20B20FR further increased plant height for both species compared to B light alone, and showed a promotion effect similar to 20FR. Treatment 20B compared to increased FW for arugula, showing a similar promotion effect as 20FR. Compared to D, B light alone or 20B20FR increased leaf thickness, but 20FR reduced leafy index. Furthermore, B light alone or 20B20FR, compared to 20FR, increased chlorophyll content index, leafy index, dry matter content, and leaf thickness to varying degrees with species. Apparantly, although 20FR, compared to B light alone, had a greater promotion effect on elongation, it showed some negative effects on microgreen quality. Plant height met the machine harvest requirement for mustard under nighttime B light treatments and for arugula under 20B20FR, but not for both species under D. Overall, there is

the potential to use monochromatic B light with or without FR light, depending on plant species, during nighttime to promote stem elongation without compromising crop yield or appearance quality.

CHAPTER FIVE

OVERNIGHT SUPPLEMENTAL BLUE, RATHER THAN FAR-RED, LIGHT IMPROVES MICROGREEN YIELD AND APPEARANCE QUALITY WITHOUT COMPROMISING NUTRITIONAL QUALITY DURING WINTER GREENHOUSE PRODUCTION[3]

ABSTRACT

To determine whether supplemental B light or FR light supplied overnight can promote microgreen elongation to facilitate machine harvesting and improve microgreen quality and yield, two common microgreen species, mustard (*B. juncea*) and arugula (*E. sativa*), were grown in a greenhouse in Guelph, Ontario, Canada during January 2019. Low intensity (14 $\mu mol \cdot m^{-2} \cdot s^{-1}$) of B or FR was applied to microgreens from 1730 HR to 0630 HR the following morning, and no supplemental lighting (D) was used as a control. After two-week of light treatments, B light promoted stem elongation by 16% and 10%, and increased crop yield by 32% and 29% in mustard and arugula, respectively, relative to the D-treatment plants. Also, B light delivered at night increased cotyledon area in mustard, leaf mass per area in arugula, and enhanced cotyledon color in both species, despite having no effects on total chlorophyll, carotenoid and phenolic concentrations. However, FR did not increase stem length or fresh weight as compared to plants treated with D, reduced plant height as compared to B light in both species, and reduced cotyledon area in arugula. Also, FR light, compared to D or B light, reduced stem diameter, and phytochemical concentrations in both species. Therefore, low-intensity B light can be applied overnight for winter greenhouse microgreen production, due to

[3] *Article citation*: Ying, Q., Y. Kong, and Y. Zheng. 2020. Overnight supplemental blue, rather than far-red, light improves microgreen yield and appearance quality without compromising nutritional quality during winter greenhouse production. HortScience. In press

its beneficial effects on appearance quality and crop yield, without negatively affecting nutritional quality.

5.1 INTRODUCTION

Microgreens are tender leafy vegetables harvested after two cotyledons have fully developed, with or without the first true leaves, and are becoming popular in the worldwide markets due to their high nutritional value (Treadwell et al., 2016; Xiao et al., 2012). In some regions that have long and cold winters like Canada, winter production of microgreens in local greenhouses has become an option. It is reported that the import value of greenhouse vegetables was much higher than the farm gate value of local greenhouse vegetables in Canada (Statistics Canada, 2016). However, it is difficult to import or transport microgreens from other regions to Canada, because they are highly perishable products (Mir et al., 2017). Moreover, microgreens have a short growth period (i.e., 7–20 d), so they can be grown with many cropping cycles in greenhouses all winter long.

The low natural light level during winter months is one of the most limiting factors in greenhouse vegetable production in northern regions, such as Canada (Demers and Gosselin, 2002). During the winter months (e.g. Nov. to Jan.), the natural daily light integral (DLI) in northern United States and southern Canada normally ranges between 5 to 15 $mol \cdot m^{-2} \cdot d^{-1}$, which corresponds to a daily average *PPFD* of 58 to 174 $\mu mol \cdot m^{-2} \cdot s^{-1}$ (Faust and Logan, 2018). The available light level in greenhouses; however, could be further reduced by 30% to 60%, due to the transmission losses through greenhouse construction and covering materials (Critten, 1993; Llewellyn et al., 2013). Therefore, in the above regions, the DLI in the greenhouses can be as low as 2 to 6 $mol \cdot m^{-2} \cdot d^{-1}$ during the winter months (i.e., the daily average *PPFD* of 23 to 69

$\mu mol \cdot m^{-2} \cdot s^{-1}$ depending on the greenhouse structure). For microgreens, the recommended minimum DLI has been elusive in the literature. However, for greenhouse vegetable (including microgreens) production in southern Canada, the yield and most quality metrics increased with increasing DLI within the range of 6.9 to 24 $mol \cdot m^{-2} \cdot d^{-1}$ (Jones-Baumgardt et al., 2020; Kong et al., 2018b; Kong and Zheng, 2019). Consequently, winter greenhouse production under low natural light conditions is a great challenge for growers, due to the decreased yield and quality of horticultural crops, including microgreens.

Supplemental lighting (SL) is a common practice for greenhouse production to deal with low natural light issues. Hofstra et al. (1969) found that low intensity supplemental light was efficient for carbon assimilation and plant growth, and around 13 $\mu mol \cdot m^{-2} \cdot s^{-1}$ supplemental light was used five times more efficiently during the night as compared to daytime in terms of CO_2 fixation. Also, overnight SL can be economically beneficial in some regions, like Ontario in Canada, where the electricity cost during night is almost half of that compared to daytime. Therefore, overnight SL may benefit crop production more efficiently. LEDs have been increasingly used as SL source in greenhouses, because of many advantages over traditional lamps (Brandon et al., 2012; Gómez et al., 2013). Among the advantages, adjustable spectral quality enables growers to control plant growth and development using LED lights based on their production purposes. However, the optimal spectral quality of LED is unclear for overnight low-intensity SL during winter greenhouse microgreen production in terms of yield and quality.

Microgreens with longer stems are normally more attractive to most consumers, so plant height is one of the most important traits of microgreen appearance quality. In addition, plant height or stem length is also an important technological quality trait. Most microgreens are

harvested with a minimum height of 5 cm (Kyriacou et al., 2016), and inhibition of stem elongation would delay harvest time and thereby extending crop cycle time. Also, commercial microgreen production has been increasingly switching from hand- to machine-harvesting to reduce labor costs. Microgreens with plant height < 5 cm are difficult for machine harvesting (communication with commercial growers). Although daytime SL can increase microgreen yield and some quality traits, it inhibits stem (or hypocotyl) elongation and causes difficulty in machine harvesting (Jones-Baumgardt et al., 2019). Therefore, it would be interesting to investigate whether stem elongation can be promoted by overnight SL without compromising yield and quality in winter greenhouse production.

Recently, our lab has found that monochromatic B light (400 nm to 500 nm), compared to R light (600 to 700 nm), promoted stem elongation of indoor-grown microgreens under LED lighting as the sole light source at a *PPFD* of around 100 or 50 $\mu mol \cdot m^{-2} \cdot s^{-1}$ and a photoperiod of both 24 and 16 h (Kong et al., 2019c; 2019b). In addition to promoting stem elongation, B light was found to reduce cotyledon size relative to R light, as well as change plant color, and increase biomass partitioning to stem, despite the similar FW of stems and leaves (Kong et al., 2019b). It is concluded that the promoted stem elongation is a shade-avoidance response mediated by B associated with low phytochrome activity, indicated by low phytochrome photostationary state (PPS; < 0.6) (Kong et al., 2018b), which may also involve a co-action among the three photoreceptors, phytochrome, cryptochrome and phototropin (Kong and Zheng, 2020). However, it is unclear whether a similar promotion effect on stem elongation associated with other responses can be found under overnight supplemental B in winter greenhouse microgreen production.

In natural light environment, enriched FR light levels can also promote stem elongation as a shade-avoidance response by lowering phytochrome equilibrium (i.e., decreasing phytochrome activity) (Demotes-Mainard et al., 2016). Increased FR level at the end of day (EOD) had been shown to enhance stem elongation in many species, including chrysanthemum (Lund et al., 2007); poinsettia (Islam et al., 2014); and tomato rootstock (Chia and Kubota, 2010). Extending the photoperiod with supplemental FR light was extremely useful to promote shoot elongation of Japanese pear during the first several months of the seedling stages (Ito et al., 2014). However, plants grown under a light environment with high FR levels might undergo some negative effects, such as decreased chlorophyll content and leaf thickness (Demotes-Mainard et al., 2016). These negative effects may potentially compromise microgreen quality.

It is unknown whether B or FR light is more effective as a source of overnight SL in promoting elongation, while having fewer negative effects on yield and other quality traits, for winter greenhouse microgreen production. Therefore, the objective of this study was to evaluate the effects of overnight SL with low-intensity B or FR LEDs, using no SL as control, on winter greenhouse production of arugula and mustard microgreens, in terms of appearance quality (including stem elongation), crop yield, and phytochemical concentrations.

5.2 MATERIALS AND METHODS

5.2.1 Greenhouse conditions and plant materials

The experiment was performed in the Edmund C. Bovey building research greenhouse at the University of Guelph, Guelph, ON (latitude 43°33’N, longitude 80°15’W) during January of 2019. Three adjacent greenhouse compartments (6.2 m × 7.6 m), with three benches oriented east to west, were used in the experiment (Fig. 5.1). Each compartment was independently

controlled using an Argus environmental control system (Argus Controls Systems Ltd., Surrey, BC, Canada) at day/night temperature and relative humidity (RH) of 21/19 °C and 70% respectively. The temperature and RH were also logged using the same system with 15-min intervals in each compartment. The natural light intensity in the greenhouse was logged by a sunlight-calibrated quantum sensor (SQ-110, Apogee Instruments, Logan, Utah, USA) tethered to the datalogger (HOBO U-12 Temp/RH/2 External Logger, Onset Computer Corp., Bourne, MA), placed on the center bench in the middle compartment, to measure natural *PPFD* data at the bench level on 5 min intervals throughout the trial. The daily variation of air temperature, RH, natural *PPFD* and DLI inside the experimental greenhouse are presented in Fig. 5.2.

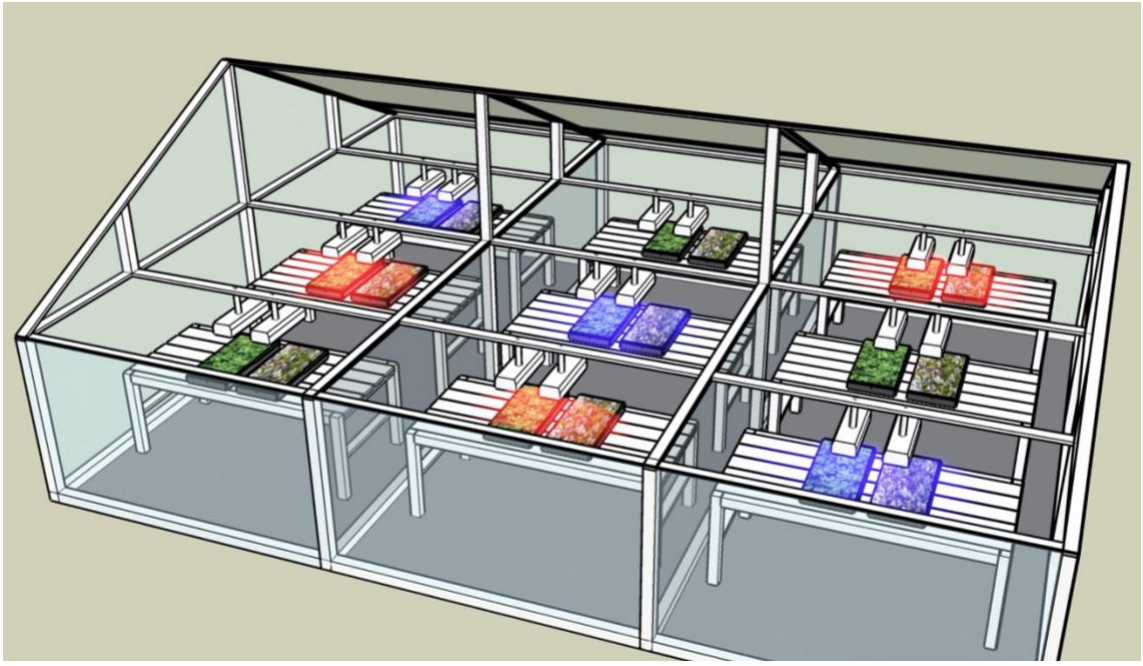


Figure 5.1. Schematic of the experimental greenhouse.

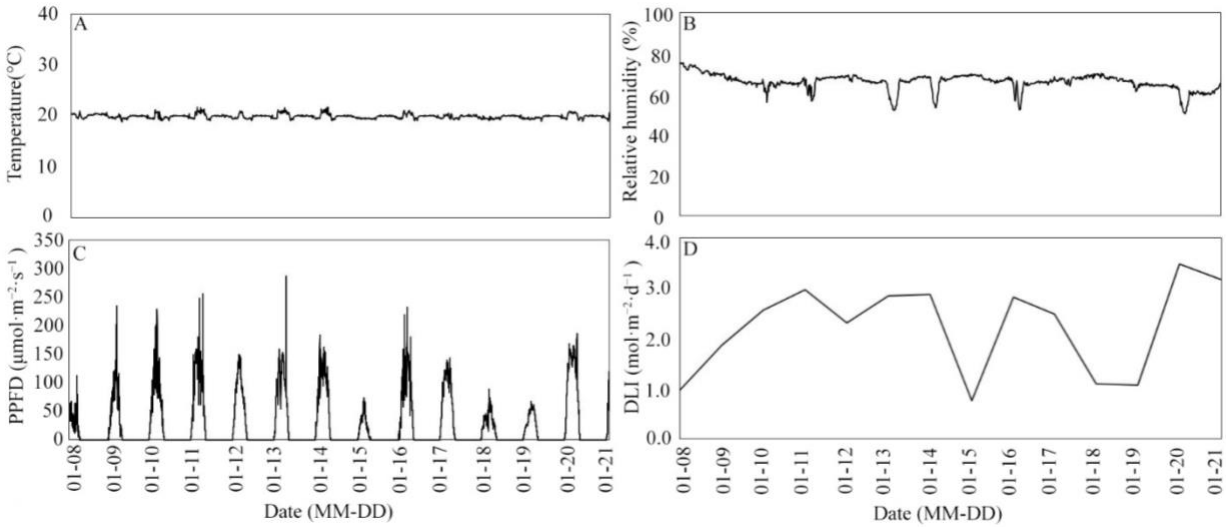


Figure 5.2. Daily variation of air temperature (A), relative humidity (B), natural photosynthetic photon flux density (C) and daily light integral (D) in the experimental greenhouse during the period of lighting treatment from 1000 HR, Jan. 8 to 1000 HR, Jan. 21 in 2019.

Seeds of mustard (*B. juncea*; Johnny's Selected Seeds, Winslow, ME, USA) and arugula (*E. sativa*; Suba Seeds Company S.P.A., Longiano, FC, Italy) microgreens were sown in fiber trays (48.5 cm × 23 cm × 3.5 cm) with pre-incorporated organic substrates from Greenbelt Microgreens Ltd. (Lynden, ON, Canada). The seeding rate was 36.4 g·m^{-2} for both arugula (1.56 mg/seed) and mustard (1.63 mg/seed). A thin layer of coconut coir was used to cover the substrates to maintain moisture. At the same day of seeding, two sown trays (one species each) were placed at the center of each greenhouse bench to start treatments. Plants were monitored daily, and top irrigated until drainage was observed.

5.2.2 Experimental design and treatments setup

For each species, randomized complete block design (3 treatments × 3 replicates) was used in the experiment (Fig. 5.3A). The three replicates (i.e., blocks) were allocated to the three

greenhouse compartments. Each compartment had three benches. In each block (i.e., compartment), three treatments were randomly assigned to three benches: 1) D: no supplemental light (SL); 2) B: supplemental 14 $\mu mol \cdot m^{-2} \cdot s^{-1}$ B LED light (400 nm –500 nm, peak at 445 nm); 3) FR: supplemental 14 $\mu mol \cdot m^{-2} \cdot s^{-1}$ far-red LED light (700 nm–800 nm, peak at 735 nm). The light spectral distributions under different SL light treatments are presented in Fig. 5.4. PPS values of night-time light treatments were calculated based on the light spectral distribution using the protocol developed by our laboratory (Mah et al., 2019) according to Sager et al. (1988). For each bench, SL was provided by one programmable LED light fixture (LX602C, Heliospectra AB, Sweden), mounted 1.35 m above the bench (measured from the bottom of the LED array). The lights were on 30 min after sunset (around 1730 HR) until 30 min before sunrise (around 0630 HR) throughout the experimental period (Fig. 5.3B). Each bench was isolated with automatic blackout that was closed/opened when the SL was on/off, to avoid neighbour lighting effects. For each bench, light measurements were taken at canopy level on a 4 × 4 square grid (i.e., at 16 different locations) centered below the light within an experimental area of 50 cm × 50 cm, using a radiometrically-calibrated spectrometer (XR-Flame, Ocean Optics, Dunedin, FL, USA) coupled to a 400 nm x 1.9 m patch cord with a CC3 cosine corrector. The measured photon flux density (PFD) of supplemental B and FR light was 13.7 ± 0.1 $\mu mol \cdot m^{-2} \cdot s^{-1}$ and 14.1 ± 0.4 $\mu mol \cdot m^{-2} \cdot s^{-1}$ (mean ± SE, n=3), respectively.

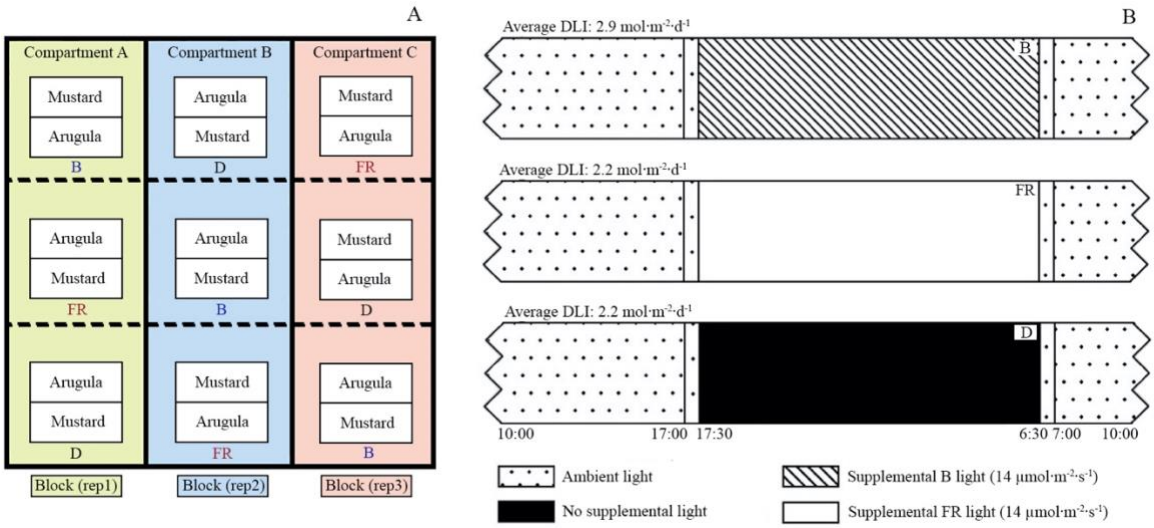


Figure 5.3. Schematic of the experimental design (A) and experimental treatments (B). B: supplemental blue LED light; D: no supplemental light; FR: supplemental far-red LED light.

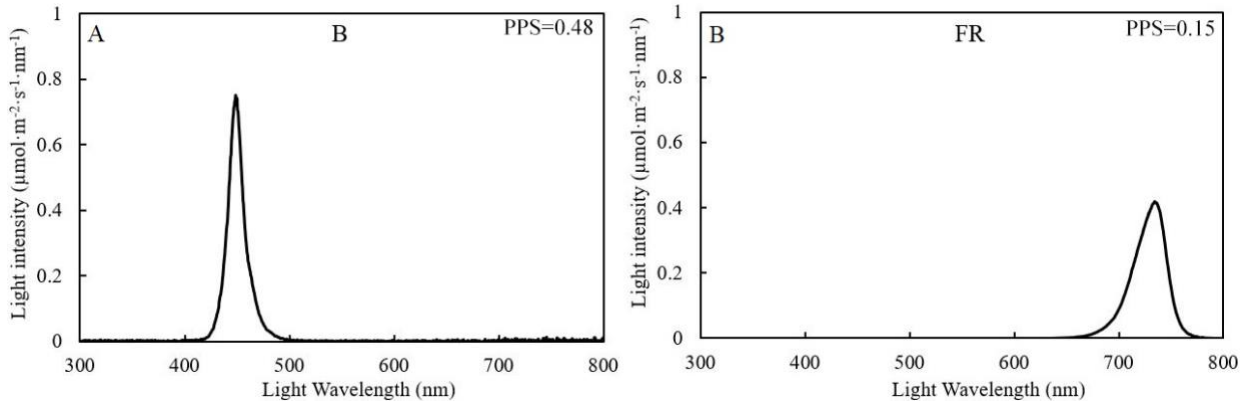


Figure 5.4. Spectral distribution and phytochrome photostationary state (PPS) of overnight supplemental blue (B, peak at 445 nm) and far-red (FR, peak at 735nm) light of 14 $\mu mol \cdot m^{-2} \cdot s^{-1}$.

5.2.3 Growth and appearance quality measurements

Both mustard and arugula microgreens were harvested 14 d after sowing, right after the first true leaf started to emerge. In each tray, three subsamples (with a substrate surface area of 76.4 cm^2 each, including plants and the substrate) of microgreens were randomly sampled

from each tray using a cylindrical core sampler, and all plants within each core were cut from the substrate level and weighed for FW. Then the harvested plants were placed into paper bags and dried at 65 °C until constant weight for DW determination. Another 15 plants were randomly sampled for measurements of plant heights with a ruler. The plant height was measured from the base of the stem to the top of cotyledon in life position. From the remainder of the plants left in each tray, three subsets of 5 plants were sampled randomly for the measurements of stem length and diameter, cotyledon area and color, and leaf mass unit area (LMA). The cotyledons were cut off from stems, and the cotyledons and stems were imaged together with a standard reference by a scanner (Canoscan LiDE 25, Canon Inc., Japan) at 600 dpi. The averaged cotyledon area, stem length and diameter of each subset (5 plants) were measured by ImageJ (version 1.42, National Institute of Health, USA). Stem diameter was measure at 1 cm above the bottom of the stem. The R, G, B values of the cotyledon color were also obtained by ImageJ, and then hue angle of cotyledon was calculated based on method of Karcher and Richardson (2003) after a calibration between the scanned and actual color using Munsell color chips (Munsell color, 2018). After scanning, the cotyledons and stems of each 5 plants were weighed separately, and also placed in paper bags and dried at 65 °C until constant weight for DW measurements. The LMA was calculated according to Eq [1].

$$\text{LMA } (g \cdot m^{-2}) = \text{Cotyledon DW / Cotyledon area} \qquad [1]$$

5.2.4 Phytochemical measurements

For each light treatment replicate, three subsamples (≈5 g FW each) of fresh plant tissue, including cotyledons, stems and first true leaves, were randomly taken from each tray, quickly frozen in liquid N_2, pulverized into fine powder with an ice-cold mortar and pestle, and

collected in a 50 mL conical tube. These flash frozen microgreen tissues were stored at –80 °C before the measurements. Unless otherwise mentioned, all chemicals required were purchased from Sigma-Aldrich Inc. (Oakville, ON, Canada).

For measurements of total chlorophyll and carotenoid, around 20 mg of each frozen sample was used. The samples were re-suspended with 1 mL ice-cold 100% methanol in 1.7 mL pre-chilled Eppendorf tubes. The samples were stored on ice under darkness after they were vortexed for 1 min twice, and then centrifuged at 13,000 *g* for 5 min at 4 °C. The supernatants were collected in new 1.7 mL pre-cooled Eppendorf tubes and serial dilutions (up to three times) in a final volume of 200 µL were prepared in 100% ice cold methanol. The methanolic extracts were transferred to a 96-well microplate reader (BioTek, Vermont, USA) for measuring absorbance (A). The A readings were at wavelength 665 nm, 652 nm and 476 nm for chlorophyll *a*, chlorophyll *b* and carotenoid, respectively. The total chlorophyll and total carotenoid concentrations were calculated using the equations from Lichtenthaler and Buschmann (2001). Each subsample was measured six times (i.e., supernatant twice, and its 1:2, 1:3 dilution twice).

Total phenolic was measured according to methods of Ainsworth and Gillespie (2007) with some modifications. Around 20 mg frozen microgreen tissues were transferred in 1.7 mL Eppendorf tube and was resuspended with 1 mL ice-cold 100% methanol, and vortexed twice for 1 min. The samples were then centrifuged at 13,000 *g* for 5 min at 4 °C. 25 µL of each sample supernatant, 1:2 dilution of the supernatants, standards, and blanks were dispensed in different wells in a 96-well microplate reader. Thereafter, 125 µl 10% Folin-Ciocalteau (F-C) reagent was added to each well, and the plate was incubated at room temperature for 10 min. Thereafter, 125 µl of 7.5% (w/v) Na_2CO_3 was added, and the A was measured at 765 nm. The

total phenolic concentrations were calculated against the gallic acid standard curve ranging from 0.018–0.6 $mg \cdot L^{-1}$.

5.2.5 Statistical analyses

Data were analyzed by SPSS statistical software (Version 25.0, IBM, New York, USA). Treatment effects were determined by one-way analysis of variance (ANOVA), and mean separations were performed using Tukey's honestly significant difference (HSD) test at $P \leq 0.05$.

5.3 RESULTS

5.3.1 Appearance quality

B light increased plant height by 16% and 10% in mustard and arugula relative to the D treatment, respectively (Fig. 5.5A). FR increased plant height by 7% in mustard as compared to the D treatment, while having no effect on arugula. For both mustard and arugula, plants grown under B light were 8% taller than those under FR light. Stem length in mustard and arugula was increased by 12% and 20% under B light relative to the D treatment respectively, but was not different between FR and D for both species (Fig. 5.5B). Also, B light increased stem length for arugula by 14% compared to FR light, but no difference was apparent for mustard.

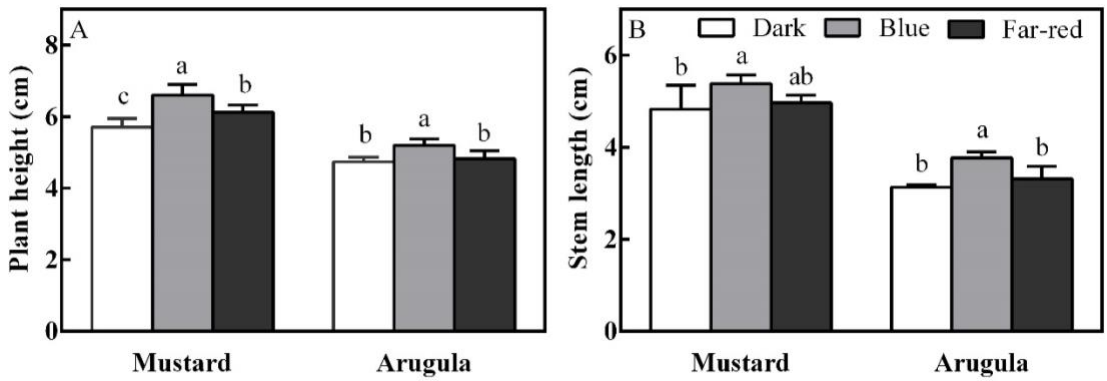


Figure 5.5. Plant height (A) and stem length (B) of mustard and arugula microgreens grown in the greenhouse with no supplemental light (D), supplemental ≈14 µmol·m^{-2}·s^{-1} blue (B) or far-red (FR) light overnight. Data are means ± SE of three experimental replicates (n = 3). For each species, bars bearing the same letter are not significantly different by Tukey's honestly significant difference test at $P \leq 0.05$.

Compared to the D treatment, B light increased cotyledon area of mustard by 22%, but did not affect the cotyledon area of arugula (Table 5.1). In contrast, FR reduced cotyledon area of arugula by 24% as compared to the D treatment. For mustard and arugula, cotyledon area was respectively 44% and 32%, greater under B light than D. There was no difference in LMA among the three treatments: B, FR and D for mustard (Table 5.1). However, LMA in arugula was 21% and 18% greater with B light as compared to D and FR, respectively, and there was no difference between the D and FR treatments. As for stem diameter, stems were thicker under B light and D than FR for both species, although no difference was observed in stem diameter under B and D for both species. Cotyledon hue angle was smaller for mustard and larger for arugula under B, compared to D and FR, while there was no difference in cotyledon hue angle between D and FR (Table 5.1). The changes in hue angle under B light relative to the D and FR

treatment indicated that the cotyledon color increased redness and greenness in mustard and arugula, respectively (Fig. 5.6).

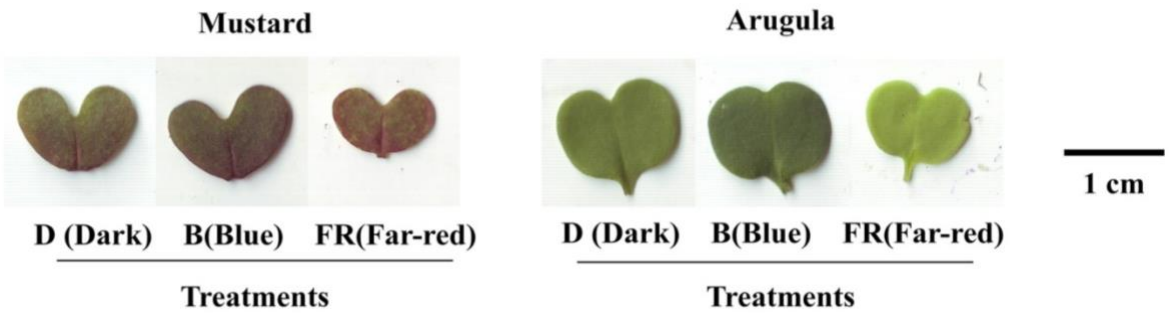


Figure 5.6. Cotyledons of mustard (left) and arugula (right) microgreens grown in the greenhouse with no supplemental light (D), supplemental ≈14 µmol·m^{-2}·s^{-1} blue (B) or far-red (FR) light overnight.

Table 5.1. Cotyledon area, leaf mass per unit area (LMA), stem diameter and hue angle of mustard and arugula microgreens grown in the greenhouse with no supplemental light (D), supplemental ≈14 µmol·m^{-2}·s^{-1} blue (B) or far-red (FR) light overnight.

Treatment	Cotyledon area (cm^2/plant)	LMA (g·m^{-2})	Stem diameter (mm)	Cotyledon hue angle (°)
		Mustard		
Dark (D)	1.1 ± 0.1 b	76.1 ± 5.5 a	0.8 ± 0.0 a	44.2 ± 4.8 a
Blue (B)	1.4 ± 0.1 a	78.3 ± 5.9 a	0.8 ± 0.0 a	28.5 ± 5.2 b
Far-red (FR)	0.9 ± 0.1 b	84.3 ± 14.0 a	0.7 ± 0.0 b	36.9 ± 3.5 a
		Arugula		
Dark (D)	1.6 ± 0.1 a	85.7 ± 3.0 b	0.8 ± 0.0 a	78.3 ± 0.5 b

Blue (B)	1.6 ± 0.1 a	104.0 ± 10.2 a	0.8 ± 0.0 a	80.0 ± 0.1 a
Far-red (FR)	1.2 ± 0.1 b	81.6 ± 7.1 b	0.7 ± 0.0 b	78.8 ± 0.5 b

Data are means ± SE of three experimental replicates (n = 3). For each species, means in the same column followed by the same letter are not significantly different by Tukey's honestly significant difference test at P ≤ 0.05.

5.3.2 Plant biomass

The FW (kg·m^{-2}) of mustard and arugula was 36% and 28% greater respectively, with B light compared to the D treatment, while FR did not affect FW of either species (Fig. 5.7A). B light increased FW by 55% and 26% in mustard and arugula, respectively, as compared to FR. There was no difference in DW among D, B, and FR for mustard (Fig. 5.7B). However, the DW of arugula was increased by 36% and 56% under B compared to D and FR, respectively, and was not changed by FR relative to D.

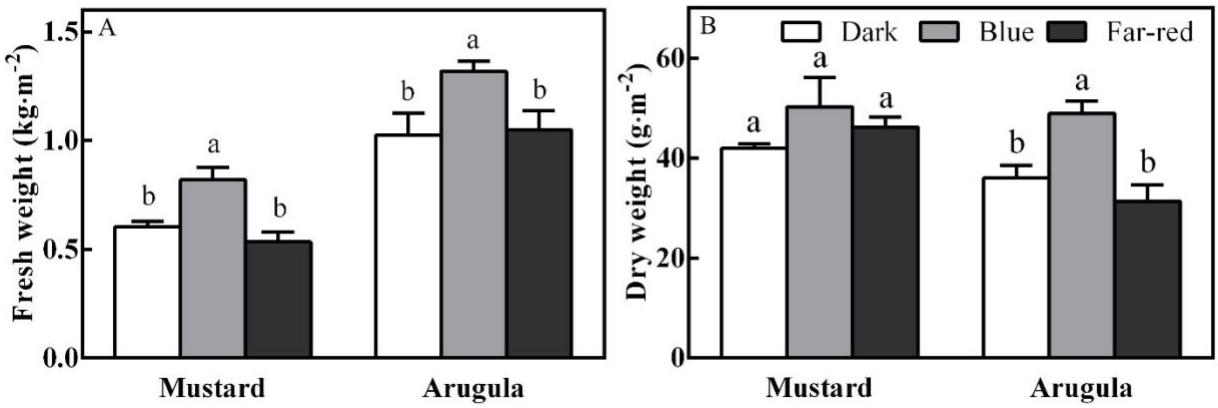


Figure 5.7. Fresh (A) and Dry (B) weight of mustard and arugula microgreens grown in the greenhouse with no supplemental light (D), supplemental ≈14 µmol·m^{-2}·s^{-1} blue (B) or far-red (FR) light overnight. Data are means ± SE of three experimental replicates (n = 3). For each

species, bars bearing the same letter are not significantly different by Tukey's honestly significant difference test at $P \leq 0.05$.

5.3.3 Phytochemical concentrations

For both species, B compared to D did not affect total chlorophyll concentration (Fig. 5.8A). However, FR compared to D reduced the total chlorophyll concentration by 30% and 31% for mustard and arugula, respectively. Also, B increased the total chlorophyll concentration by 52% for arugula compared to FR, but showed no difference for mustard.

Total carotenoid concentration was not affected by B as compared to D for both species, but was reduced by 23% under FR as compared to D for arugula (Fig. 5.8B). B light increased total carotenoid concentration by 42% and 35% for mustard and arugula, respectively, relative to FR.

For both species, the total phenolic concentration was similar between B light and D treatments, whereas FR reduced these levels by 23% and 33% for mustard and arugula, respectively, as compared to D (Fig. 5.8C).The total phenolic concentration was 39% higher for arugula with B compared to FR light, but was not different for mustard.

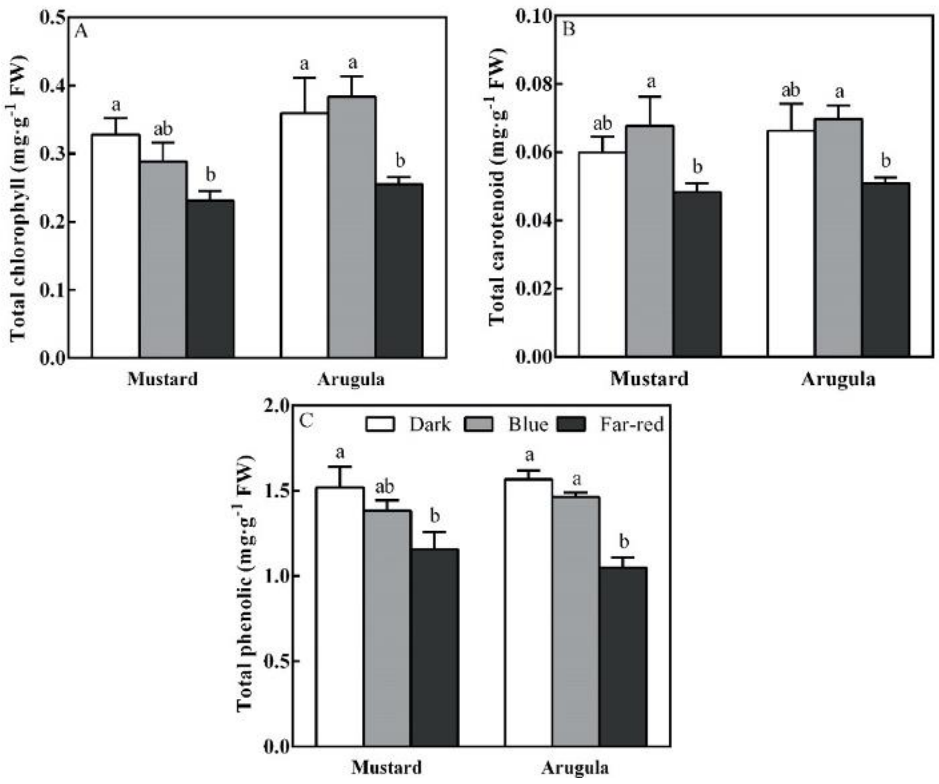


Figure 5.8. Total chlorophyll, carotenoid, and phenolic concentration of mustard and arugula microgreens grown in the greenhouse with no supplemental light (D), supplemental ≈14 $\mu mol \cdot m^{-2} \cdot s^{-1}$ blue (B) or far-red (FR) light overnight. Data are means ± SE of three experimental replicates (n = 3). For each species, bars bearing the same letter are not significantly different by Tukey's honestly significant difference test at $P \leq 0.05$.

5.4 DISCUSSION

5.4.1 Overnight supplemental B improves appearance quality compared to D, showing better effects than supplemental FR.

For indoor microgreen production using LED lighting as sole light source, 50–100 $\mu mol \cdot m^{-2} \cdot s^{-1}$ B light promoted stem elongation to meet the demands of machine harvesting (Kong et al., 2019b). For controlled environment production of chrysanthemums, night-time

supplemental B LED of 20 or 100 μmol·m^{-2}·s^{-1} promoted stem length (Jeong et al., 2014; Nissim-Levi et al., 2019). Similarly, for winter greenhouse microgreen production, overnight supplemental 14 μmol·m^{-2}·s^{-1} B LED light increased plant height in both species relative to the D treatment, culminating in taller plants than the machine-harvest requirement (i.e. 5 cm). Monochromatic B LED can be used as either sole-source or overnight supplemental light to promote microgreen elongation, and thus facilitate machine harvesting. Although the detailed pathway involved in B light-induced stem elongation is still unclear, recent studies indicate that deactivated cryptochrome and activated phototropin (i.e., two B light photoreceptors), in addition to phytochrome, may also contribute to this process (Huché-Thélier et al., 2016; Kong et al., 2018a; Kong and Zheng, 2020; Pashkovskiy et al., 2016). Interestingly, in the present study, overnight supplemental FR light did not promote stem elongation for either species, and increased plant height only for mustard possibly due to promoted petiole elongation. It appears that overnight supplemental B vs. FR light has a greater promotion effect on microgreen elongation, which is also supported by the increased plant height for both species.

The result from the present study is somewhat contrasting to another one of our microgreen studies performed in a growth chamber using a LED combination of R and B light ($B_{20}R_{80}$) as daytime light source (Ying et al, 2020c), where night-time FR light had a greater effect on promoting elongation than B light. The difference between the two studies might have resulted from the different phytochrome activities before starting supplemental B or FR light. In growth chamber, combination of R and B LEDs used for daytime lighting had a relatively high PPS value (i.e., ≈0.88), and might have maintained a high phytochrome activity which could be maintained for several hours even when light was off (Gaba and Black, 1979). In greenhouse, the

natural light at the end of day normally causes a low phytochrome equilibrium, due to a low R:FR ratio (Smith, 1982) (e.g., R:FR = 0.6, and PPS= 0.48 in our experimental greenhouse, unpublished data). It has been proven that the effect of B light on plant elongation varies with change of phytochrome equilibrium induced by mixing with other wavelengths (e.g., R light) at low-level. High phytochrome equilibrium (e.g., PPS > 0.6) leads to an inhibition of B while low phytochrome equilibrium (e.g., PPS <0.6) leads to a promotion effect of B (Kong et al., 2018a; 2019a).

Supplemental B light also increased cotyledon area and redness in mustard, as well as cotyledon thickness (i.e., LMA) and greenness for arugula. The changes in these traits would be more appealing to the consumers, and could potentially increase microgreens appearance quality based on our communication with commercial growers. In contrast, supplemental FR light reduced cotyledon area, color, and stem diameter for both species compared to D, and reduced LMA for arugula, and cotyledon area and stem diameter for both species compared to B light. Obviously, supplemental FR light also induced some typical responses to shade in other traits (Smith and Whitelam, 1997), despite showing little promotion effect on plant elongation. Pure FR light indicates a deep shade signal, which may make plants to switch to other responses (e.g., decreased leaf greenness through reducing chlorophyll content) to adapt to the deep shade other than elongation to capture light, in which phytochrome A is known to play a role (Yang et al., 2018a; Gommers et al., 2013; Barnes et al., 1996). Considering the negative effects of FR light on the above plant traits, supplemental B light seems to have better effects on microgreens appearance quality.

5.4.2 Overnight supplemental B, rather than FR, promotes plant biomass accumulation in microgreens.

Supplemental B light (around 30% of natural DLI; average at 2.2 mol·m^{-2}·d^{-1}) increased FW per unit area (i.e., crop yield) for both species, but supplemental FR light did not. Also, supplemental B light increased crop FW for both species relative to the FR light. It appears that overnight supplemental B, rather than FR, can promote fresh biomass accumulation in microgreens during winter greenhouse production. Similar promotion effects have also been found in growth chamber studies. Under daytime fluorescent light of 3.6 mol·m^{-2}·d^{-1}, overnight supplemental B light of 50 µmol·m^{-2}·s^{-1} for 14 h increased the shoot FW by 26% to 54% of chrysanthemum, mustard, and onion (*Allium cepa* L.) compared to no SL, while supplemental FR did not affect or reduced the FW (Sase et al., 2012). In the growth chamber under R and B LED light of 17.3 mol·m^{-2}·d^{-1}, supplemental B light of 20 µmol·m^{-2}·s^{-1} increased the FW of arugula but not mustard microgreens (Ying et al., 2020c). The above studies showed the potential of using supplemental B light to increase FW, although the efficiency of the promotion was affected by daytime light condition, supplemental light intensity, duration and also plant species. The higher yield of microgreens under supplemental B compared to D or FR, in the present study, was also supported by the modified appearance quality traits such as longer and thicker stems, or larger and thicker cotyledons. The increased yield under overnight supplemental B will potentially benefit microgreen production, since these crops are sold on a FW basis.

In addition to FW, supplemental B light increased DW per unit growing area for arugula, but supplemental FR did not increased DW in either species. Also, supplemental B light

increased DW for arugula relative to FR. The normalized DW for DLI measurement was comparable across all the treatments. Nevertheless, it appears that overnight supplemental B, rather than FR light, could promote dry biomass accumulation in some microgreen species (i.e., arugula). The increased DW under supplemental B, rather than FR light in the present study might have resulted from the SL-increased photo-assimilate accumulation, since B falls in the range of *PAR*, but FR does not. Many studies have shown the synergistic effects of FR light in increasing biomass when it supplies with *PAR* (Zhen and Bugbee, 2020), but the biomass was not increased when FR was applied alone in present study. Moreover, the contribution of photosynthetic assimilates to microgreen biomass may vary among species with different seed size. During the short growth period of microgreens, plants mainly experience a transition from heterotrophic to autotrophic growth, and photosynthesis contributes less to plant biomass than seed reservation for larger- vs. smaller-sized species (Jones-Baumgardt et al., 2019, 2020). In the present study, mustard had a larger seed size than arugula (1.63 vs. 1.56 mg/seed), which may partly explain why supplemental B, compared to D or supplemental FR, did not increased DW in mustard, but in arugula.

5.4.3 Overnight supplemental B does not reduce phytochemical concentrations compared to D, but supplemental FR does

It has been documented that higher B light levels can increase phytochemical contents in plants. For example, chlorophyll content (g per unit leaf area) increased when B was added to R light from 0% to 50% in cucumber seedlings (Hogewoning et al., 2010). Total carotenoid concentration increased in beet (*Beta vulgaris* L.) microgreen when B light was added to R light

from 0% up to 33% (Samuolienė et al., 2017). Chlorophyll content (SPAD value) and total phenolic concentration in 'Sunmang' lettuce increased when adding B to R from 0% up to 47% (Son and Oh, 2013). However, in the present study, supplemental B did not affect the total chlorophyll, carotenoid, or phenolic concentration ($mg \cdot g^{-1}$ FW) in either species compared to D. Possibly, in the previous studies, B acted together with the other wavelength (e.g., R light), but in the present study, B was applied alone during the night time and at very low level. It has been proved that B light mediates phytochemical (e.g., anthocyanin) synthesis mainly through cryptochrome, which requires active phytochrome for full expression, and this requirement can be supplied by low levels of R (Ahmad and Cashmore, 1997). Another explanation for the inconsistency between the previous studies and ours is that the responses to B light might vary among species or even cultivars. A previous greenhouse study on pak choi (*B. oleracea*) indicated that daytime supplemental 50 $\mu mol \cdot m^{-2} \cdot s^{-1}$ of B light, compared to no SL, did not increase the total chlorophyll, carotenoid, or phenolic contents in green-leafed cultivars, but increased those phytochemical contents in red-leafed cultivars (Zheng et al., 2018).

In the present study, supplemental FR reduced the concentrations of phytochemicals (i.e., total chlorophyll, carotenoid and phenolic) in microgreens compared to D, or supplemental B light, in most cases. Lower chlorophyll content (either unit leaf area or unit FW) was found in petunia (*Petunia axilaris*) plants treated with FR light compared to R light at the end of photoperiod (Casal et al., 1987). Supplementing FR light to cool white fluorescent light induced plants to express diverse shade-avoidance syndrome, such as decreasing the contents of chlorophyll and carotenoid, which was corresponding with decreasing phytochrome equilibrium (Kalaitzoglou et al., 2019). The biosynthesis of chlorophyll is negatively regulated by

Phytochrome Interacting Factor 1 (PIF 1), which activity is greatly affected by phytochrome photoequilibrium (Huq et al., 2004). Also, FR radiation, as a light competition signal, can elicit a down-regulation of plant chemical defenses (e.g., reduced biosynthesis of phenolic) (Moreno et al., 2009). This may partly explain the decrease in total phenolic concentration under supplemental FR light in the present study.

5.5 CONCLUSION

In summary, during winter greenhouse production (with a natural DLI of around 2.2 $mol \cdot m^{-2} \cdot d^{-1}$ at canopy level), overnight supplemental 14 $\mu mol \cdot m^{-2} \cdot s^{-1}$ B light, compared to D or FR light, improved microgreen appearance quality demonstrated by increased plant height (essential for machine harvest), stem length, cotyledon area, LMA, or cotyledon coloring, varying with species. Also, B light compared to the D treatment, increased microgreen yield, but FR vs. D did not. Furthermore, FR light vs. D or B light reduced the concentrations of phytochemicals (i.e., total chlorophylls, carotenoids and phenolics). Therefore, overnight supplemental low-intensity B light, rather than FR light, is beneficial to winter greenhouse production of microgreens.

CHAPTER SIX

GENERAL DISCUSSION AND CONCLUSIONS

Microgreens with high yield, enriched phytochemical content and a minimum height of 5 cm are desirable for their commercial production. Previous studies have indicated that light quality greatly affects plant growth and development. However, the responses vary greatly across species and growing stages. The results of this thesis study demonstrated the opportunities of using light strategies to induce desired morphological responses with fewer trade-off in terms of biomass and phytochemical contents. The outcomes of this research will enable commercial growers to manipulate the appearance, growth and phytochemical contents of specific microgreen species efficiently by customizing the LED light spectra.

In chapter 2 and 3, the effects of B and R light ratio from LED lighting on the growth, yield, appearance quality, and phytochemical and nitrate contents of *Brassicaceae* microgreens (kale, cabbage, arugula and mustard) were investigated, under the same *PPFD* of 300 $\mu mol \cdot m^{-2} \cdot s^{-1}$ and 16 h photoperiod. The FW and DW were generally not affected by different B and R light ratios, except cabbage, which showed quadratic (peaking at 15%) responses in FW. Increasing B light percentages inhibited the hypocotyl elongation and cotyledon area of kale and mustard, but did not affect arugula and cabbage in these two traits. Cotyledons were darker red for mustard and less pure green for the other three species under higher B light percentage. Phytochemical analyses indicated that total phenolic, anthocyanin content, and ascorbate metabolites could be increased by increasing B light percentages. The increase in these antioxidant compositions potentially improve the quality of microgreen and provides distinct benefit to human health. However, the magnitude of the effect on morphological and phytochemical response varied

across species. Therefore, species-specific lighting recipes need to be considered to meet specific cultivation requirements.

In chapter 4 &5, the effects of nighttime supplemental B and/or FR light on microgreen (arugula and mustard) growth, quality and phytochemical content were evaluated. In the growth chamber with daytime BR LED lighting of 300 $\mu mol \cdot m^{-2} \cdot s^{-1}$ for 16h, 8 h nighttime supplemental B light treatments promoted stem elongation, increased cotyledon area and FW compared to no supplemental light, although the enhancements varied with microgreen species and B light regimens. Although plant height was also promoted under supplemental FR light, it led to decrease of relative chlorophyll content and cotyledon thickness. Similarly in greenhouse, overnight B light at 14 $\mu mol \cdot m^{-2} \cdot s^{-1}$ efficiently promoted plant height and hypocotyl length, increased FW for both arugula and mustard, increased cotyledon area, leaf mass per area for arugula or mustard, without decreasing any phytochemical (i.e., total chlorophylls, carotenoids and phenolics) accumulation compared to dark. However, overnight FR light at the same photon flux density reduced stem diameter and phytochemical contents compared to dark, without having a positive effect on stem elongation or biomass accumulation compared to dark. Therefore, low-intensity monochromatic B light could be applied during nighttime, as an effective way to promote stem elongation, without compromising other growth metrics or phytochemical content.

Overall, the results from Chapter 2, 3, 4 and 5 presented the profound capability of modulating plant growth, appearance and phytochemical content by altering light spectra from LED light, during daytime or nighttime. This thesis highlighted the use of B LED light, including higher percentage in RB-LED lighting and supplementing during nighttime, in promoting

phytochemical content and stem elongation. However, there are still knowledge gaps that need to be filled and other interesting research questions need to be answered.

The underlying biological mechanisms of LED light spectral quality regulating plant morphology and secondary metabolism need further studies. This thesis reported that the antioxidant compositions (i.e., ascorbate, phenolic and anthocyanin) were increased under increased B light proportions. However, in-depth knowledge on the light regulated phytochemical biosynthetic pathway is needed, key enzymes and gene expression that are affected by light stimulus need to be explored. Also, our results showed that monochromatic B light promoted stem elongation, which is somehow contradictory to the previous understanding that B light induced compact plants. Therefore, investigating phytohormone content and metabolism in plant tissues would provide better understanding of responses to the spectrum-associated regulatory effects. A detailed protocol for further research on investigating the phytohormone and phytochemical contents regulated by B and R LED light is provided in Appendix I.

From a production point of view, the commercially available LED lights also contain wavelengths other than the combination of R and B light. More studies could be conducted exploring the light recipes including wavelengths such as UV, G, and amber light on growth, yield and phytochemical contents of microgreens, in order to 'optimize' their production. Also, more research is needed for other commercialized microgreen species and cultivars, given the great variations of growth, yield and quality of microgreens in responding to the changing light qualities found in this thesis study. All the studies were conducted under controlled environment where other environmental factors (e.g., light intensity, photoperiod,

temperature, CO_2 and RH) were fixed. Changes in these environmental factors could interact with light spectral quality to affect plant growth and development, which need further investigation. In the greenhouse trial, nighttime low intensity B light was efficient to promote stem elongation and yield of microgreens during wintertime. However, the efficiency of nighttime supplemental light needs to be tested during summertime in the future, when the DLI was substantially higher than winter.

LITERATURE CITED

Agati, G, E. Azzarello, S. Pollastri, and M. Tattini. 2012. Flavonoids as antioxidants in plants: Location and functional significance. Plant Sci. 196: 67–76.

Ahmad, M. and A.R. Cashmore. 1997. The blue-light receptor cryptochrome 1 shows functional dependence on phytochrome A or phytochrome B in *Arabidopsis thaliana*. Plant J. 11:421–427.

Ainsworth, E. a and K.M. Gillespie. 2007. Estimation of total phenolic content and other oxidation substrates in plant tissues using Folin-Ciocalteu reagent. Nat. Protoc. 2:875–877.

Ali, M.B., L. Khandaker, S. Oba. 2009. Comparative study on functional components, antioxidant activity and color parameters of selected colored leafy vegetables as affected by photoperiods. J. Food, Agric. Environ. 7:392–398.

Alrifai, O., X. Hao, M.F. Marcone, and R. Tsao. 2019. Current review of the modulatory effects of LED lights on photosynthesis of secondary metabolites and future perspectives of microgreen vegetables. J. Agric. Food Chem. 67:6075–6090.

ASTM, 2003. Standard tables for reference solar spectral irradiances: direct normal and hemispherical on 37° tilted surface. http://www.astm.org.

Atta, M., A. Idris, A. Bukhari, and S. Wahidin. 2013. Intensity of blue LED light: A potential stimulus for biomass and lipid content in fresh water microalgae *Chlorella vulgaris*. Bioresour. Technol. 148:373–378.

Barnes, S.A., N.K. Nishizawa, R.B. Quaggio, G.C. Whitelam, and N.H. Chua. 1996. Far-red light blocks greening of arabidopsis seedlings via a phytochrome A-mediated change in plastid development. Plant Cell 8:601–615.

Barta, D.J., T.W. Tibbitts, R.J. Bula, and R.C Morrow. 1992. Evaluation of light emitting diode characteristics for a space-based plant irradiation source. Adv. Sp. Res. 12:141–149.

Bergstrand, K.J.I. 2017. Methods for growth regulation of greenhouse produced ornamental pot-and bedding plants: A current review. Folia Hort. 29:63–74.

Bian, Z.H., Q.C. Yang, and W.K. Liu. 2015. Effects of light quality on the accumulation of phytochemicals in vegetables produced in controlled environments: A review. J. Sci. Food Agric. 95:869–877.

Björkman, M., I. Klingen, A.N.E. Birch, A.M. Bones, T.J.A. Bruce, T.J. Johansen, R. Meadow, J. Mølmann, R. Seljåsen, L.E. Smart and D. Stewart. 2011. Phytochemicals of Brassicaceae in plant protection and human health – Influences of climate, environment and agronomic practice. Phytochemistry 72:538–556.

Bouly, J.P., E. Schleicher, M. Dionisio-Sese, F. Vandenbussche, D. Van Der Straeten, N. Bakrim, S. Meier, A. Batschauer, P. Galland, R. Bittl, and M. Ahmad. 2007. Cryptochrome blue light photoreceptors are activated through interconversion of flavin redox states. J. Biol. Chem. 282:9383–9391.

Bourget, C.M. 2008. An introduction to light-emitting diodes. HortScience 43:1944–1946.

Brandon, M.F., N. Lu, T. Yamaguchi, M. Takagaki, T. Maruo, T. Kozai, and W. Yamori. 2012. Next evolution of agriculture: A review of innovations in plant factories. Handb. Photosynth. 723–740.

Brazaitytė, A., S. Sakalauskienė, G. Samuolienė, J. Jankauskienė, A. Viršilė, A. Novičkovas, R. Sirtautas, J. Miliauskienė, V. Vaštakaitė, L. Dabašinskas, and P. Duchovskis. 2015. The

effects of LED illumination spectra and intensity on carotenoid content in *Brassicaceae* microgreens. Food Chem. 173:600–606.

Brazaityte, A., A. Viršile, J. Jankauskiene, S. Sakalauskiene, G. Samuoliene, R. Sirtautas, A. Novičkovas, L. Dabašinskas, J. Miliauskiene, V. Vaštakaite, A. Bagdonavičiene, and P. Duchovskis. 2015. Effect of supplemental UV-A irradiation in solid-state lighting on the growth and phytochemical content of microgreens. Int. Agrophysics 29:13–22.

Brown, C.S., A.C. Schuerger, and J.C. Sager. 1995. Growth and photomorphogenesis of pepper plants under red light-emitting diodes with supplemental blue or far-red lighting. J. Am. Soc. Hortic. Sci. 120:808–813.

Casal, J.J. and M.A. Mazzella. 1998. Conditional synergism between cryptochrome 1 and phytochrome B is shown by the analysis of *phyA*, *phyB*, and *hy4* simple, double, and triple mutants in Arabidopsis. Plant Physiol. 118:19–25.

Casal, J.J., P.J. Aphalo, and R.A. Sanchez. 1987. Phytochrome effects on leaf growth and chlorophyll content in *Petunia axilaris*. Plant. Cell Environ. 10:509–514.

Carvalho, S.D., and Folta, K.M., 2014. Sequential light programs shape kale (*Brassica napus*) sprout appearance and alter metabolic and nutrient content. Hortic. Res. 1:1–13.

Chang, S., C. Li, X. Yao, S. Chen, X. Jiao, X. Liu, Z. Xu, and R. Guan. 2016. Morphological, photosynthetic, and physiological responses of rapeseed leaf to different combinations of red and blue lights at the rosette stage. Front. Plant Sci. 7:1–12.

Chen, H., X. Tong, L. Tan, and L. Kong. 2020. Consumers' acceptability and perceptions toward the consumption of hydroponically and soil grown broccoli microgreens. J. Agric. Food Chem. 2: 100051.

Cheynier, V., G. Comte, K.M. Davies, V. Lattanzio, and S. Martens. 2013. Plant phenolics: Recent advances on their biosynthesis, genetics, and ecophysiology. Plant Physiol. Biochem. 72:1–20.

Chia, P.L. and C. Kubota. 2010. End-of-day far-red light quality and dose requirements for tomato rootstock hypocotyl elongation. HortScience 45:1501–1506.

Cogdell, R. J., and A.T. Gardiner. 1993. [18] Functions of carotenoids in photosynthesis. In Methods in enzymology. 214: 185-193

Comar, C.L. and F.P. Zscheile. 1942. Analysis of plant extracts for chlorophylls a and b by a photoelectric spectrophotometric method. Plant Physiol. 17:198–209.

Cope, K.R., and B. Bugbee. 2013. Spectral effects of three types of white light-emitting diodes on plant growth and development: Absolute versus relative amounts of blue light. HortScience 48:504–509.

Critten, D.L. 1993. A review of the light transmission into greenhouse crops. Acta Hort. 328:9–32.

Darko, E., P. Heydarizadeh, B. Schoefs, and M.R. Sabzalian. 2014. Photosynthesis under artificial light: the shift in primary and secondary metabolism. Philos. Trans. R. Soc. B Lond. B Biol. Sci. 369:20130243.

Davis, P.A. and C. Burns. 2016. Photobiology in protected horticulture. Food Energy Secur. 5:223–238.

Demers, D.A. and A. Gosselin. 2002. Growing greenhouse tomato and sweet pepper under supplemental lighting: Optimal photoperiod, negative effects of long photoperiod and their causes. Acta Hort. 580:83–88.

Demotes-Mainard, S., T. Péron, A. Corot, J. Bertheloot, J. Le Gourrierec, S. Pelleschi-Travier, L. Crespel, P. Morel, L. Huché-Thélier, R. Boumaza, A. Vian, V. Guérin, N. Leduc, and S. Sakr. 2016. Plant responses to red and far-red lights, applications in horticulture. Environ. Expt. Bot. 121:4–21.

Di Gioia, F., C. Mininni, and P. Santamaria. 2015. How to grow microgreens. p.51-81. In: F. Di Gioia and P. Santamaria (eds.). Microgreens-Novel fresh and functional food to explore all the value of biodiversity. Italy: ECO-logica srl. Bari, Italy.

Długosz-Grochowska, O., R. Wojciechowska, M. Kruczek, and A. Habela. 2017. Supplemental lighting with LEDs improves the biochemical composition of two *Valerianella locusta* (L.) cultivars. Hort. Environ. Biotechnol. 58:441–449.

Dou, H., G. Niu, M. Gu, and J. Masabni. 2017. Effects of light quality on growth and phytonutrient accumulation of herbs under controlled environments. Horticulturae 3:36.

Dougher, T.A., and B. Bugbee. 2001. Differences in the response of wheat, soybean and lettuce to reduced blue radiation. Photochem. Photobiol. 73:199–207.

Emerson, R., R. Chalmers and C. Cederstrand. 1957. Some factors influencing the longwave limit of photosynthesis. Proc Natl Acad Sci U S A 43: 133–143

European Union. 2011. Commission Regulation (EU) No 1258/2011 of 2 December 2011 amending Regulation (EC) No 1881/2006 as regards maximum levels for nitrates in foodstuffs. Off. J. Eur. Union 320:15–17.

Faust, J.E. 2002. First Research Report. Light management in greenhouses. II. Plant Growth Responses to Daily Light Integrals. Available from http://www.specmeters.com/assets/1/7/A051.pdf

Faust, J.E. and J. Logan. 2018. Daily light integral: A research review and high-resolution maps of the United States. HortScience 53:1250–1257.

Flaherty, E.J., B. Lum, J.R. Deell, S. Subedi, B.J. Shelp, and G.G. Bozzo. 2018. Metabolic alterations in postharvest pear fruit as influenced by 1-methylcyclopropene and controlled atmosphere storage. J. Agric. Food Chem. 66:12989–12999.

Folta, K.M., and K.S. Childers. 2008. Light as a growth regulator: Controlling plant biology with narrow-bandwidth solid-state lighting systems. HortScience 43:1957–1964.

Fukuda, N., C. Ajima, T. Yukawa, and J.E. Olsen. 2016. Antagonistic action of blue and red light on shoot elongation in petunia depends on gibberellin, but the effects on flowering are not generally linked to gibberellin. Environ. Expt. Bot. 121:102–111.

Fukuda, N., Y. Ishii, H. Ezura, and J.E. Olsen. 2011. Effects of light quality under red and blue light emitting diodes on growth and expression of FBP28 in petunia. Acta Hortic. 907:361–366.

Gaba, V. and M. Black. 1979. Two separate photoreceptors control hypocotyl growth in green seedlings. Nature 278:51–54.

Gerovac, J.R., J.K. Craver, J.K. Boldt, and R.G. Lopez. 2016. Light intensity and quality from sole-source light-emitting diodes impact growth, morphology, and nutrient content of *Brassica* microgreens. HortScience 51:497–503.

Giliberto, L., 2005. Manipulation of the blue light photoreceptor cryptochrome 2 in tomato affects vegetative development, flowering time, and fruit antioxidant content. Plant Physiol. 137:199–208.

Giovannoni, J.J. 2007. Completing a pathway to plant vitamin C synthesis. Proc. Natl. Acad. Sci. U. S. A. 104:9109–9110.

Goins, G.D., N.C. Yorio, M.M. Sanwo, and C.S. Brown. 1997. Photomorphogenesis, photosynthesis, and seed yield of wheat plants grown under red light-emitting diodes (LEDs) with and without supplemental blue lighting. J. Exp. Bot. 48:1407–1413.

Gómez, C., R.C. Morrow, C.M. Bourget, G.D. Massa, and C.A. Mitchell. 2013. Comparison of intracanopy light-emitting diode towers and overhead high-pressure sodium lamps for supplemental lighting of greenhouse-grown tomatoes. HortTechnology 23:93–98.

Gommers, C.M.M., E.J.W. Visser, K.R.S. Onge, L.A.C.J. Voesenek and R. Pierik. 2013. Shade tolerance: When growing tall is not an option. Trends Plant Sci. 18:65–71.

Gonçalves, B., A.P. Silva, J. Moutinho-Pereira, E. Bacelar, E. Rosa, and A.S. Meyer. 2007. Effect of ripeness and postharvest storage on the evolution of colour and anthocyanins in cherries (*Prunus avium* L.). Food Chem. 103:976–984.

Graham, T., N. Yorio, P. Zhang, G. Massa, and R. Wheeler. 2019. Early seedling response of six candidate crop species to increasing levels of blue light. Life Sci. Sp. Res. 21:40–48.

Griffiths, M., W.R. Sistrom,G. Cohen-Bazire and Stanier, R.Y. Function of carotenoids in photosynthesis. Nature. 175:1211-1214.

Guo, H., H. Yang, T.C. Mockler, and C. Lin. 1998. Regulation of photoperiodic flowering by *Arabidopsis* photoreceptors. Science. 279:1360–1363.

Hachiya, T. and Y. Okamoto. 2017. Simple spectroscopic determination of nitrate, nitrite, and ammonium in *Arabidopsis thaliana*. Bio-protocol 7:1–13.

Hata, N., Y. Hayashi, E. Ono, H. Satake, A. Kobayashi, T. Muranaka, and A. Okazawa. 2013. Differences in plant growth and leaf sesamin content of the lignan-rich sesame variety "Gomazou" under continuous light of different wavelengths. Plant Biotechnol. 30:1–8.

Heo, J.W., D.H. Kang, H.S. Bang, S.G. Hong, C. Chun, and K.K. Kang. 2012. Early growth, pigmentation, protein content, and phenylalanine ammonia-lyase activity of red curled lettuces grown under different lighting conditions. Korean J. Hort. Sci. Technol. 30:6–12.

Hernández, R. and C. Kubota. 2014. Growth and morphological response of cucumber seedlings to supplemental red and blue photon flux ratios under varied solar daily light integrals. Sci. Hortic. 173:92–99.

Hernández, R. and C. Kubota. 2016. Physiological responses of cucumber seedlings under different blue and red photon flux ratios using LEDs. Environ. Expt. Bot. 121:66–74.

Ho, C. 1992. Phenolic compounds in food: An overview, p.2-7. In: Phenolic compounds in food and their effects on health I. ACS Symposium Series, American Chemical Society, DC.

Hoenecke, M.E., R.J. Bula, and T.W. Tibbitts. 1992. Importance of "blue" photon levels for lettuce seedlings grown under red-light-emitting diodes. HortScience 27:427–430.

Hoffmann, A.M., G. Noga, and M. Hunsche. 2016. Alternating high and low intensity of blue light affects PSII photochemistry and raises the contents of carotenoids and anthocyanins in pepper leaves. Plant Growth Regul. 79:275–285.

Hofstra, G., G. JA Ryle, and R. Williams. 1969. Effects of extending the day length with low-intensity light on the growth of wheat and cocksfoot. Aust. J. Biol. Sci. 22:333.

Hogewoning, S.W., G. Trouwborst, H. Maljaars, H. Poorter, W. van Ieperen, and J. Harbinson. 2010. Blue light dose-responses of leaf photosynthesis, morphology, and chemical

composition of *Cucumis sativus* grown under different combinations of red and blue light. J. Exp. Bot. 61:3107–3117.

Hu, F.B. 2003. Plant-based foods and prevention of cardiovascular disease: An overview. Am. J. Clin. Nutr. 78:544–551.

Huché-Thélier, L., L. Crespel, J. L. Gourrierec, P. Morel, S. Sakr, N. Leduc. 2016. Light signaling and plant responses to blue and UV radiations-Perspectives for applications in horticulture. Environ. Exp. Bot. 121:22–38.

Huq, E., B. Al-Sady, M. Hudson, C. Kim, K. Apel, P.H. Quail. 2004. Phytochrome-interacting factor 1 is a critical bHLH regulator of chlorophyll biosynthesis. Biol. Sci. Collect. 305: 1937–1941.

Ilakiya, T., E. Parameswari, V. Davamani, and E. Prakash. 2020. Microgreens - Combacting malnutrition problem. Res. Today 2:110–112.

Islam, M.A., D. Tarkowská, J.L. Clarke, D.R. Blystad, H.R. Gislerød, S. Torre, and J.E. Olsen. 2014. Impact of end-of-day red and far-red light on plant morphology and hormone physiology of poinsettia. Sci. Hort. 174:77–86.

Ito, A., T. Saito, T. Nishijima, and T. Moriguchi. 2014. Effect of extending the photoperiod with low-intensity red or far-red light on the timing of shoot elongation and flower-bud formation of 1-year-old Japanese pear (*Pyrus pyrifolia*). Tree Physiol. 34:534–546.

Jabben, M., C. Beggs, and E. Schaufer. 1982. Dependence of Pfr/Ptot-ratios on light quality and light quantity. Photochem. Photobiol. 35:709-712.

Jahns, P. and A.R. Holzwarth. 2012. The role of the xanthophyll cycle and of lutein in photoprotection of photosystem II. Biochim. Biophys. Acta Bioenerg. 1817:182–193.

Jeong, S.W., S.W. Hogewoning, and W. van Ieperen. 2014. Responses of supplemental blue light on flowering and stem extension growth of cut chrysanthemum. Sci. Hort. 165:69–74.

Johnson, R.E., Y. Kong, and Y. Zheng. 2020. Elongation growth mediated by blue light varies with light intensities and plant species: A comparison with red light in arugula and mustard seedlings. Environ. Expt. Bot. 169:103898.

Jones-Baumgardt, C., D. Llewellyn, Q. Ying, and Y. Zheng. 2019. Intensity of sole-source light-emitting diodes affects growth, yield, and quality of Brassicaceae microgreens. HortScience. 54:1168–1174.

Jones-Baumgardt, C., D. Llewellyn, and Y. Zheng. 2020. Different microgreen genotypes have unique growth and yield responses to intensity of supplemental PAR from light-emitting diodes during winter greenhouse production in southern Ontario. HortScience. 55:156-163.

Kadomura-Ishikawa, Y., K. Miyawaki, S. Noji, and A. Takahashi. 2013. Phototropin 2 is involved in blue light-induced anthocyanin accumulation in *Fragaria* × *ananassa* fruits. J. Plant Res. 126:847–857.

Kaiser, C., and M. Ernst. 2018. Microgreens. Center for Crop Diversification, University of Kentucky College of Agriculture, Food and Environ. http://www. uky. edu/ccd/sites/www. uky.edu.ccd/files/microgreens.pdf.

Kalaitzoglou, P., W. Van Ieperen, J. Harbinson, M. Van Der Meer, and M.C. Rousseaux. 2019. Effects of continuous or end-of-day far-red light on tomato plant growth, morphology, light absorption, and fruit production. Front. Plant Sci. 10:1–11.

Karcher, D.E., and M.D. Richardson. 2003. Quantifying turfgrass color using digital image analysis. Crop Sci. 43:943–951.

Kim, E.Y., S.A. Park, B.J. Park, Y. Lee, and M.M. Oh. 2014. Growth and antioxidant phenolic compounds in cherry tomato seedlings grown under monochromatic light-emitting diodes. Hort. Environ. Biotechnol. 55:506–513.

Kim, S.J., E.J. Hahn, J.W. Heo, and K.Y. Paek. 2004. Effects of LEDs on net photosynthetic rate, growth and leaf stomata of chrysanthemum plantlets in vitro. Sci. Hortic. 101:143–151.

Kong, Y., D. Kamath, and Y. Zheng. 2019b. Blue versus red light can promote elongation growth independent of photoperiod: A study in four *Brassica* microgreens species. HortScience. 54:1955–1961.

Kong, Y., D. Llewellyn, and Y. Zheng. 2018b. Response of growth, yield, and quality of pea shoots to supplemental light-emitting diode lighting during winter greenhouse production. Can. J. Plant Sci. 740:732–740.

Kong, Y., K. Schiestel, and Y. Zheng. 2019a. Pure blue light effects on growth and morphology are slightly changed by adding low-level UVA or far-red light: A comparison with red light in four microgreen species. Environ. Expt. Bot. 157:58–68.

Kong, Y., K. Schiestel, and Y. Zheng. 2019c. Maximum elongation promoted as a shade-avoidance response by blue light is related to deactivated phytochrome: A comparison with red light in four microgreen species. Can. J. Plant Sci. 2019. 100: 314-326

Kong, Y., M. Stasiak, M.A. Dixon, and Y. Zheng. 2018a. Blue light associated with low phytochrome activity can promote elongation growth as shade-avoidance response: A comparison with red light in four bedding plant species. Environ. Expt. Bot. 155:345–359.

Kong, Y. and Y. Zheng. 2019. Response of growth, yield, and quality of edible-podded snow peas to supplemental LED lighting during winter greenhouse production. Can. J. Plant Sci. 99:676–687.

Kong, Y. and Y. Zheng. 2020. Phototropin is partly involved in blue-light-mediated stem elongation, flower initiation, and leaf expansion: A comparison of phenotypic responses between wild *Arabidopsis* and its phototropin mutants. Environ. Exp. Bot. 171:103967.

Kopsell, D.A., C.E. Sams, T.C. Barickman, and R.C. Morrow. 2014. Sprouting broccoli accumulate higher concentrations of nutritionally important metabolites under narrow-band light-emitting diode lighting. J. Am. Soc. Hortic. Sci. 139:469–477.

Kozai, T. 2013. Plant factories in Japan - Current situation and prespectives. Chronica Hort. 53:8–11.

Kreslavski, V.D., D.A. Los, F.J. Schmitt, S.K. Zharmukhamedov, V.V. Kuznetsov, and S.I. Allakhverdiev. 2018. The impact of the phytochromes on the photosynthetic processes. Biochim. Biophys. Acta Bioenerg. 1859:400-408.

Kyriacou, M.C., Y. Rouphael, F. Di, A. Kyratzis, F. Serio, M. Renna, S. De Pascale, and P. Santamaria. 2016. Micro-scale vegetable production and the rise of microgreens. Trends Food Sci. Technol. 57:103–115.

Kubota, C., P. Chia, Z. Yang, and Q. Li. 2012. Applications of far-red light emitting diodes in plant production under controlled environments. Acta Hort. 952:59–66.

Landschoot, P.J., and C.F. Mancino. 2000. A comparison of visual vs. instrumental measurement of color differences in bentgrass turf. HortScience 35:914–916.

Lee, K., and B. Lee. 2013. Estimation of rice growth and nitrogen nutrition status using color digital camera image analysis. Eur. J. Agron. 48:57–65.

Legris, M., Y.Ç. Ince, and C. Fankhauser. 2019. Molecular mechanisms underlying phytochrome-controlled morphogenesis in plants. Nat. Commun. 10:5219

Li, H., C. Tang, and Z. Xu. 2013. The effects of different light qualities on rapeseed (*Brassica napus* L.) plantlet growth and morphogenesis in vitro. Sci. Hortic. 150:117–124.

Li, Q. and C. Kubota. 2009. Effects of supplemental light quality on growth and phytochemicals of baby leaf lettuce. Environ. Exp. Bot. 67:59–64.

Li, Y., Y. Zheng, H. Liu, Y. Zhang, Y. Hao, S. Song, and B. Lei. 2019. Effect of supplemental blue light intensity on the growth and quality of Chinese kale. Hort. Environ. Biotechnol. 60:49–57.

Lichtenthaler, H.K. and C. Buschmann. 2001. Chorophylls and carotenoids: measurement and characterization by UV-VIS spectroscopy. Food Anal. Chemistry 1:F4.3.1-F4.3.8.

Lin, C., and D. Shalitin. 2003. Cryptochrome structure and signal transduction. Annu. Rev. Plant Biol. 54:469–496.

Liu, B., Z. Yang, A. Gomez, B. Liu, C. Lin, and Y. Oka. 2016. Signaling mechanisms of plant cryptochromes in *Arabidopsis thaliana*. J. Plant Res. 129:137–148.

Liu, X., Y. Li, and S. Zhong. 2017. Interplay between light and plant hormones in the control of *Arabidopsis* seedling chlorophyll biosynthesis. Front. Plant Sci. 8:1–6.

Liu, Y., S. Fang, W. Yang, X. Shang, and X. Fu. 2018. Light quality affects flavonoid production and related gene expression in *Cyclocarya paliurus*. J. Photochem. Photobiol. B Biol. 179:66–73.

Llewellyn, D., Y. Zheng, and M. Dixon. 2013. Survey of how hanging baskets influence the light environment at lower crop level in ornamental greenhouses in Ontario, Canada. HortTechnology 23:823–829.

Lobiuc, A., V. Vasilache, O. Pintilie, T. Stoleru, M. Burducea, M. Oroian, and M.M. Zamfirache. 2017. Blue and red LED illumination improves growth and bioactive compounds contents in acyanic and cyanic *Ocimum basilicum* L. microgreens. Molecules 22:2111.

Lund, J.B., T.J. Blom, and J.M. Aaslyng. 2007. End-of-day lighting with different red/far-red ratios using light-emitting diodes affects plant growth of *Chrysanthemum × morifolium* Ramat. "Coral Charm." HortScience 42:1609–1611.

Ma, G., L. Zhang, C.K. Setiawan, K. Yamawaki, T. Asai, F. Nishikawa, S. Maezawa, H. Sato, N. Kanemitsu, and M. Kato. 2014. Effect of red and blue LED light irradiation on ascorbate content and expression of genes related to ascorbate metabolism in postharvest broccoli. Postharvest Biol. Technol. 94:97–103.

Macknight, R.C., W.A Laing, S.M. Bulley, R.C. Broad, A.A.T Johnson, and R.P. Hellens. 2017. Increasing ascorbate levels in crops to enhance human nutrition and plant abiotic stress tolerance. Curr. Opin. Biotechnol. 44:153-160.

Maevskaya, S.N. and N.G. Bukhov. 2005. Effect of light quality on nitrogen metabolism of radish plants. Russ. J. Plant Physiol. 52:304–310.

Mah, J.J., D. Llewellyn, Y. Zheng. 2019. Protocol for converting spectrometer radiometric data to photon flux units [Microsoft Excel Spreadsheet]. Guelph, University of Guelph. Available from: http://www.ces.uoguelph.ca/TechNotes.shtml.

Marschner, H. 1995. Mineral Nutrition of Higher Plants. Academic Press, London.

Massa, G.D., H.H. Kim, R.M. Wheeler, and C.A. Mitchell. 2008. Plant productivity in response to LED lighting. HortScience 43:1951–1956.

Matsuda, R., K. Ohashi-Kaneko, K. Fujiwara, E. Goto, and K. Kurata. 2004. Photosynthetic characteristics of rice leaves grown under red light with or without supplemental blue light. Plant Cell Physiol. 45:1870–1874.

Matsuda, R., K. Ohashi-Kaneko, K. Fujiwara, and K. Kurata. 2007. Analysis of the relationship between blue-light photon flux density and the photosynthetic properties of spinach (*Spinacia oleracea* L.) leaves with regard to the acclimation of photosynthesis to growth irradiance. Soil Sci. Plant Nutr. 53:459–465.

McCree, K.J., 1972. The action spectrum, absorptance and quantum yield of photosynthesis in crop plants. Agric. Meteorol. 9:191–216.

Meng, Q., E.S. Runkle, 2019. Far-red radiation interacts with relative and absolute blue and red photon flux densities to regulate growth, morphology, and pigmentation of lettuce and basil seedlings. Sci. Hort. 255:269–280.

Meng, Q., N. Kelly, E.S. Runkle. 2019. Substituting green or far-red radiation for blue radiation induces shade avoidance and promotes growth in lettuce and kale. Environ. Exp. Bot. 162: 383–391.

Mensinga, T.T., G.J.A. Speijers, and J. Meulenbelt. 2003. Health implications of exposure to environmental nitrogenous compounds. Toxicol. Rev. 22:41–51.

Mir, S.A., M.A. Shah, and M.M. Mir. 2017. Microgreens: Production, shelf life, and bioactive components. Crit. Rev. Food Sci. Nutr. 57:2730–2736.

Moe, R., S. O. Grimstad, and H.R. Gislerød. 2006. The use of artificial light in year round production of greenhouse crops in Norway. Acta Hortic. 711:35–42.

Möglich, A., X. Yang, R.A. Ayers and K. Moffat. 2010. Structure and function of plant photoreceptors. Annu. Rev. Plant Biol. 61:21–47

Morrow, R.C. 2008. LED lighting in horticulture. HortScience 43:1947–1950.

Murakami, K., R. Matsuda, and K. Fujiwara. 2016. Interaction between the spectral photon flux density distributions of light during growth and for measurements in net photosynthetic rates of cucumber leaves. Physiol. Plant. 158:213–224.

Nanya, K., Y. Ishigami, S. Hikosaka, and E. Goto. 2012. Effects of blue and red light on stem elongation and flowering of tomato seedlings. Acta Hortic. 956:261–266.

Naznin, M.T., M. Lefsrud, V. Gravel, and M.O.K. Azad. 2019. Blue light added with red LEDs enhance growth characteristics, pigments content, and antioxidant capacity in lettuce, spinach, kale, basil, and sweet pepper in a controlled environment. Plants 8:1–12.

Nelson, J.A. and B. Bugbee. 2014. Economic analysis of greenhouse lighting: Light emitting diodes vs. high intensity discharge fixtures. PLoS One. 9:e99010.

Neff, M.M., and E. Van Volkenburgh. 1994. Light-stimulated cotyledon expansion in *Arabidopsis* seedlings (The role of phytochrome B). Plant Physiol. 104:1027–1032.

Nhut, D.T., L.T.A. Hong, H. Watanabe, M. Goi, and M. Tanaka. 2002. Growth of banana plantlets cultured in vitro under red and blue light-emitting diode (LED) irradiation source. Acta Hortic. 575:117–124.

Nhut, D.T., T. Takamura, H. Watanabe, K. Okamoto, and M. Tanaka. 2003. Responses of strawberry plantlets cultured in vitro under superbright red and blue light-emitting diodes (LEDs). Plant Cell. Tissue Organ Cult. 73:43–52.

Nishikimi, M., R. Fukuyama, S. Minoshima, N. Shimizu, and K. Yagi. 1994. Cloning and chromosomal mapping of the human nonfunctional gene for L-gulono-gamma-lactone oxidase, the enzyme for L-ascorbic acid biosynthesis missing in man. J. Biol. Chem. 269:13685–13688.

Nissim-Levi, A., M. Kitron, Y. Nishri, R. Ovadia, I. Forer, and M. Oren-Shamir. 2019. Effects of blue and red LED lights on growth and flowering of *Chrysanthemum morifolium*. Sci. Hort. 254:77–83.

Ohashi-Kaneko, K., M. Takase, N. Kon, K. Fujiwara, and K. Kurata. 2007. Effect of light quality on growth and vegetable quality in leaf lettuce, spinach and komatsuna. Environ. Control Biol. 45:189–198

Olle, M., and A. Viršile. 2013. The effects of light-emitting diode lighting on greenhouse plant growth and quality. Agric. Food Sci. 22:223–234.

Owen, W.G., and R.G. Lopez. 2015. Customizing crop foliage color with LEDs: Ornamental crops. Greenh. Grow. 33:76–80.

Pashkovskiy, P.P., A.V. Kartashov, I.E. Zlobin, S.I. Pogosyan, V.V. Kuznetsov. 2016. Blue light alters miR167 expression and microRNA-targeted auxin response factor genes in *Arabidopsis thaliana* plants. Plant Physiol. Biochem. 104:146–154.

Pinto, E., A.A. Almeida, A.A. Aguiar, and I.M.P.L.V.O. Ferreira. 2015. Composition and analysis comparison between the mineral profile and nitrate content of microgreens and mature lettuces. J. Food Compos. Anal. 37:38–43.

Queval, G. and G. Noctor. 2007. A plate reader method for the measurement of NAD, NADP, glutathione, and ascorbate in tissue extracts: Application to redox profiling during *Arabidopsis* rosette development. Anal. Biochem. 363:58–69.

Rao, A.V. and L.G. Rao. 2007. Carotenoids and human health. Pharmacol. Res. 55:207–216.

Rispail, N., P. Morris, and K.J. Webb. 2005. Phenolic compounds: Extraction and analysis. Lotus japonicus handbook. Springer.

Roepke, J. and G.G. Bozzo. 2015. *Arabidopsis thaliana* β-glucosidase BGLU15 attacks flavonol 3-*O*-β-glucoside-7-*O*-α-rhamnosides. Phytochemistry 109:14–24.

Sæbø, A., T. Krekling, and M. Appelgren. 1995. Light quality affects photosynthesis and leaf anatomy of birch plantlets *in vitro*. Plant Cell. Tissue Organ Cult. 41:177–185.

Sá Junior, J.J. de M., A.R. Backes, D.R. Rossatto, R.M. Kolb, and O.M. Bruno. 2011. Measuring and analyzing color and texture information in anatomical leaf cross sections: An approach using computer vision to aid plant species identification. Botany-Botanique 89:467–479.

Sager, J.C., W.O. Smith, J.L. Edwards, and K.L. Cyr. 1988. Photosynthetic efficiency and phytochrome photoequilibria determination using spectral data. Trans. ASAE 31:1882–1889.

Samuolienė, G., A. Brazaitytė, A. Viršilė, S. Jankauskienė, J. Sakalauskienė, and P. Duchovskis. 2016. Red light-dose or wavelength-dependent photoresponse of antioxidants in herb microgreens. PLoS One:1–11.

Samuolienė, G., A. Viršilė, A. Brazaitytė, J. Jankauskienė, S. Sakalauskienė, V. Vaštakaitė, A. Novičkovas, A. Viškelienė, and P. Duchovskis. 2017. Blue light dosage affects carotenoids and tocopherols in microgreens. Food Chem. 228:50–56.

Samuolienė, G., A. Brazaitytė, J. Jankauskienė, A. Viršilė, R. Sirtautas, A. Novičkovas, S. Sakalauskienė, J. Sakalauskaitė, and P. Duchovskis. 2013. LED irradiance level affects growth and nutritional quality of *Brassica* microgreens. Cent. Eur. J. Biol. 8: 1241–1249.

Sase, S., C. Mito, and L. Okushima. 2012. Effect of overnight supplemental lighting with different spectral LEDs on the growth of some leafy vegetables. Acta Hort. 956:327–334.

Schwend, T., D. Prucker, and H. Mempel. 2015. Red light promotes compact growth of sunflowers. European J. Hort. Sci. 80:56–61.

Shimizu, H., Z. Ma, M. Douzono, S. Tazawa, E.S. Runkle, and R.D. Heins. 2006. Blue light inhibits stem elongation of chrysanthemum. Acta Hortic. 711:363–367.

Siegelman, H.W. and S.B. Hendricks. 1958. Photocontrol of anthocyanin synthesis in apple skin. Plant Physiol. 33:185–190.

Singh, D., C. Basu, M. Meinhardt-Wollweber, and B. Roth. 2015. LEDs for energy efficient greenhouse lighting. Renew. Sustain. Energy Rev. 49:139–147.

Smith, H. 1982. Light quality, photoperception, and plant strategy. Annu. Rev. plant Physiol. 33:481–518.

Smith, H. and G.C. Whitelam. 1997. The shade avoidance syndrome: Multiple responses mediated by multiple phytochromes. Plant, Cell Environ. 20:840–844.

Smith, H. and M.G. Holmes. 1977. The function of phytochrome in the natural environment—III. Measurement and calculation of phytochrome photoequilibria. Photochem. Photobiol. 25:547–550.

Snowden, M.C., K.R. Cope, and B. Bugbee. 2016. Sensitivity of seven diverse species to blue and green light: Interactions with photon flux. PLoS One 11:1–32.

Son, K.H., and M.M. Oh. 2013. Leaf shape, growth, and antioxidant phenolic compounds of two lettuce cultivars grown under various combinations of blue and red light-emitting diodes. HortScience 48:988–995.

Son, K.H., and M.M. Oh. 2015. Growth, photosynthetic and antioxidant parameters of two lettuce cultivars as affected by red, green, and blue light-emitting diodes. Hortic. Environ. Biotechnol. 56:639–653.

Stanley, L. and Y.W. Yuan. 2019. Transcriptional regulation of carotenoid biosynthesis in plants: So many regulators, so little consensus. Front. Plant Sci. 10:1–17.

Statistics Canada. 2016. Statistical overview of the Canadian greenhouse vegetable industry.

Stutte, G.W. 2009. Light-emitting diodes for manipulating the phytochrome apparatus. HortScience 44:231–234.

Taiz, L., E. Zeiger, I.M. Møller, and A. Murphy. 2015. Plant physiology and development. Sixth edit. Sinauer Associates.

Tanaka, R., K. Kobayashi, and T. Masuda. 2011. Tetrapyrrole metabolism in *Arabidopsis thaliana*. Arab. B. 9:e0145.

Tanaka, M., T. Takamura, H. Watanabe, M. Endo, T. Yanagi, and K. Okamoto. 1998. In vitro growth of *Cymbidium* plantlets cultured under superbright red and blue light-emitting diodes (LEDs). J. Hortic. Sci. Biotechnol. 73:39–44.

Tang, Y. and J. Liesche. 2017. The molecular mechanism of shade avoidance in crops: How data from *Arabidopsis* can help to identify targets for increasing yield and biomass production. J. Integr. Agr. 16:1244–1255.

Tarakanov, I., O. Yakovleva, I. Konovalova, G. Paliutina, and A. Anisimov. 2012. Light-emitting diodes: On the way to combinatorial lighting technologies for basic research and crop production. Acta Hortic. 956:171–178.

Terashima, I., T. Fujita, T. Inoue, W.S. Chow, and R. Oguchi. 2009. Green light drives leaf photosynthesis more efficiently than red light in strong white light: Revisiting the enigmatic question of why leaves are green. Plant Cell Physiol. 50:684–697.

Thoma, F., A. Somborn-Schulz, D. Schlehuber V. Keuter and G. Deerberg. 2020. Effects of light on secondary metabolites in selected leafy greens: A review. Front. Plant Sci. 11:497

Treadwell, D.D., R. Hochmuth, L. Landrum, and W. Laughlin. 2016. Microgreens: A new specialty crop. Univ. Florida IFAS Ext. Bul. HS1164.

Trouwborst, G., S.W. Hogewoning, O. van Kooten, J. Harbinson, and W. van Ieperen. 2016. Plasticity of photosynthesis after the ‘red light syndrome’ in cucumber. Environ. Exp. Bot. 121:75–82.

United States Department of Agriculture. 2014. Specialty greens pack a nutritional punch. Agric. Res.10–11.

Vom Endt, D., J.W. Kijne, and J. Memelink. 2002. Transcription factors controlling plant secondary metabolism: What regulates the regulators? Phytochemistry 61:107–114.

Wang, H. and X. Deng. 2004. Phytochrome signaling mechanism. Am. Soc. Plant Biol. e0074:1–28.

Wang, J., Z. Zhang, and R. Huang. 2013. Regulation of ascorbic acid synthesis in plants. Plant Signal. Behav. 8:18–20.

Wang, X.Y., X.M. Xu, and J. Cui. 2015. The importance of blue light for leaf area expansion, development of photosynthetic apparatus, and chloroplast ultrastructure of *Cucumis sativus* grown under weak light. Photosynthetica 53:213–222.

Warrington, I.J. and R.A. Norton. 1991. An evaluation of plant growth and development under various daily quantum integrals. J. Amer. Soc. Hort. Sci. 116:544–551.

Wen, B., C. Li, X. Fu, D. Li, L. Li, X. Chen, H. Wu, X. Cui, X. Zhang, H. Shen, W. Zhang, W. Xiao, and D. Gao. 2019. Effects of nitrate deficiency on nitrate assimilation and chlorophyll synthesis of detached apple leaves. Plant Physiol. Biochem. 142:363–371.

Wollaeger, H.M. and E.S. Runkle. 2015. Growth and acclimation of impatiens, salvia, petunia, and tomato seedlings to blue and red light. HortScience 50:522–529.

Xiao, Z., G.E. Lester, Y. Luo, and Q. Wang. 2012. Assessment of vitamin and carotenoid concentrations of emerging food products: Edible microgreens. J. Agr. Food Chem. 60:7644–7651.

Xiao, Z., S.R. Rausch, Y. Luo, J. Sun, L. Yu, Q. Wang, P. Chen, L. Yu, and J.R. Stommel. 2019. Microgreens of Brassicaceae: Genetic diversity of phytochemical concentrations and antioxidant capacity. LWT-Food Sci. Technol. 101:731–737.

Xu, F., S. Cao, L. Shi, W. Chen, X. Su, and Z. Yang. 2014. Blue light irradiation affects anthocyanin content and enzyme activities involved in postharvest strawberry fruit. J. Agric. Food Chem. 62:4778–4783.

Yang, C., F. Xie, Y. Jiang, Z. Li, X. Huang, and L. Li. 2018. Phytochrome A negatively regulates the shade avoidance response by increasing auxin/indole acidic acid protein stability. Dev. Cell 44:29-41.

Yabuta, Y., T. Mieda, M. Rapolu, A. Nakamura, T. Motoki, T. Maruta, K. Yoshimura, T. Ishikawa, and S. Shigeoka. 2007. Light regulation of ascorbate biosynthesis is dependent on the photosynthetic electron transport chain but independent of sugars in *Arabidopsis*. J. Exp. Bot. 58:2661–2671.

Yamaguchi, S. 2008. Gibberellin metabolism and its regulation. Annu. Rev. Plant Biol. 59:225–251.

Yee, L. 2002. Glorious greens. Restaurants Inst. 112:38-44.

Yeh, N. and J.P. Chung. 2009. High-brightness LEDs-energy efficient lighting sources and their potential in indoor plant cultivation. Renew. Sustain. Energy Rev. 13:2175–2180.

Ying, Q., Y. Kong, and Y. Zheng. 2020a. Growth and appearance quality of four microgreen species under light-emitting diode lights with different spectral combinations. HortScience. In press.

Ying, Q., Y. Kong, C. Jones-Baumgardt, and Y. Zheng. 2020b. Responses of yield and appearance quality of four *Brassicaceae* microgreens to varied blue light proportion in red and blue light-emitting diodes lighting. Sci. Hort. 259:1168-1174.

Ying, Q., Y. Kong, and Y. Zheng. 2020c. Applying blue light alone, or in combination with far-red light, during nighttime increases elongation without compromising yield and quality of indoor-grown microgreens. HortScience 55:876-881.

Ying, Q., Y. Kong, and Y. Zheng. 2020d. Overnight supplemental blue, rather than far-red, light improves microgreen yield and appearance quality without compromising nutritional quality during winter greenhouse production. HortScience. In press.

Yorio, N.C., G.D. Goins, H.R. Kagie, R.M. Wheeler, and J.C. Sager. 2001. Improving spinach, radish, and lettuce growth under red light- emitting diodes (LEDs) with blue light supplementation. Hortscience 36:380–383.

Yuan, L., Y. Yuan, J. Du, J. Sun, and S. Guo. 2012. Effects of 24-epibrassinolide on nitrogen metabolism in cucumber seedlings under $Ca(NO_3)_2$ stress. Plant Physiol. Biochem. 61:29–35.

Zha, L., W. Liu, Q. Ying, Y. Zhang, C. Zhou, and M. Shao. 2020. Regulation of ascorbate accumulation and metabolism in lettuce by the red:blue ratio of continuous light using LEDs. Front. Plant Sci. 11:704.

Zhang, H. and R. Tsao. 2016. Dietary polyphenols, oxidative stress and antioxidant and anti-inflammatory effects. Curr. Opin. Food Sci. 8:33–42.

Zhang, T., Y. Shi, F. Piao, and Z. Sun. 2018. Effects of different LED sources on the growth and nitrogen metabolism of lettuce. Plant Cell. Tissue Organ Cult. 134:231–240.

Zhang, Y.J., R.Y. Gan, S. Li, Y. Zhou, A.N. Li, D.P. Xu, and H.B. Li. 2015. Antioxidant phytochemicals for the prevention and treatment of chronic diseases. Molecules 20: 21138–21156.

Zhao, J., L.C. Davis, and R. Verpoorte. 2005. Elicitor signal transduction leading to production of plant secondary metabolites. Biotechnol. Adv. 23:283–333.

Zhen, S. and B. Bugbee, 2020. Far-red photons have equivalent efficiency to traditional photosynthetic photons: Implications for redefining photosynthetically active radiation. Plant Cell Environ. 1–14.

Zhen, S. and M.W. van Iersel. 2017. Far-red light is needed for efficient photochemistry and photosynthesis. J. Plant Physiol. 209:115–122.

Zheng, Y., Y. Zhang, H. Liu, Y. Li, Y. Liu, Y. Hao, and B. Lei. 2018. Supplemental blue light increases growth and quality of greenhouse pak choi depending on cultivar and supplemental light intensity. J. Integr. Agric. 17:2245–2256

APPENDIX I

Protocol: Investigating the phytohormone and flavonoid contents in Brassicaceae microgreens under monochromatic blue and red light, correlated with stem elongation

Background

Microgreens are a new category of vegetable crops that are harvested before or right after the emerge of first true leaves, in 7-20 d (Treadwell et al., 2016). They are gaining popularity because of their potent flavor, various color and higher nutrient contents compared to mature plants (Treadwell et al., 2016; Xiao et al., 2012). Micorgreens from *Brassicaceae* family have been intensively cultivated and studied over the past two decades, because they are rich in fibre, minerals and secondary metabolites (Björkman et al., 2011). For microgreen production, light strategies have been investigated to promote their stem elongation to meet the requirement for machine harvest, therefore saving time and labor cost for commercial growers (Kong et al., 2019a; Ying et al., 2020).

Plant growth and development are regulated by various environmental factors, among which light is the most important one (Smith, 1982). The changing light environment is perceived by photoreceptors, a group of pigments in plants, which absorb photon within certain wavelength and use the signal to initiate the photoresponses (Taiz et al., 2015). The changes of red (R, 600-700 nm) light and far-red (FR, 700-800 nm) light are perceived by phytochromes, which regulate many aspects of pant development including germination, de-etiolation, and hypocotyl elongation etc. (Wang and Deng, 2004). Cryptochromes are blue (B, 400–500nm)/UV-A (315–400 nm) light photoreceptors that involve in seed germination, plant

architecture, flower induction and secondary metabolite syntheses etc.(Fantini and Facella, 2020). Stem elongation is a typical shade avoidance response that plants exhibit increased stem elongation triggered by multiple canopy signals, particularly light signal like increased level of far-red light (FR) relative to red light (R) and decreased photosynthetically active radiation (Franklin and Whitelam, 2005).

Promoted elongation effect has also been reported by many studies under monochromatic B light in various species, compared to monochromatic R light, RB-LED, white LED and other light sources like fluorescent light under the same photosynthetic photon flux density (*PPFD*) with photoperiod ranging from 12-24 h (listed in Table 1). Recent studies from our lab showed that the elongation effect is a shade avoidance response that is associated with low phytochrome activity (Kong et al., 2018b). Moreover, other blue light photoreceptors are also involved in regulating the elongation effect, like cryptochrome and phototropin (Kong and Zheng, 2020). Although the mechanism underlying the elongation effect has not been clearly established, it is plausible that the common B light responses, like cryptochrome induced inhibition of elongation, may not be not fully activated in the absence of activated phytochrome (Hernández and Kubota, 2016; Neff and Chory, 1998).

Table 1. The effect of monochromatic blue light on stem elongation

Species and growth stage	Monochromatic B photon flux density ($\mu mol \cdot m^{-2} \cdot s^{-1}$)	Photoperiod (h)	Results of stem elongation	Reference
Cucumber seedlings	100	18	Hypocotyl length was 69% greater than monochromatic R LED, and 346% greater than under 75B:25R	(Hernández and Kubota, 2016)

Calibrachoa cuttings	40 or 80	16	Greater shoot length compared to R LED, white and B, R and white mixed fixture	(Olschowski et al., 2016)
Green perilla after transplanting	150 with different B wavelength peaks	16	Plants were taller under all B treatments compared to fluorescent light, and shorter B wavelength induced taller plants compared to longer B wavelengths	(Lee et al., 2014)
Marigold and salvia after transplanting	90	16	Longer stem than white and monochromatic R LED and fluorescent lamp	(Heo et al., 2002)
Petunia after transplanting	70/100	14/12	Higher stem length than monochromatic R LED	(Fukuda et al., 2011; 2016)
Petunia, Calibrachoa, Geranium, Marigold seedlings after transplanting	50/ 100	Continuous lighting	Promoted stem elongation compared to monochromatic R and combinations of B and R combined LEDs; 50 $\mu mol \cdot m^{-2} \cdot s^{-1}$ promoted stem elongation to a greater degree than 100 $\mu mol \cdot m^{-2} \cdot s^{-1}$ for petunia and calibrachoa	(Kong et al., 2018b)
Cabbage, Kale, Arugula and Mustard seedlings	50/100	24	Promoted stem elongation compared to monochromatic R LEDs under 50 and 100 $\mu mol \cdot m^{-2} \cdot s^{-1}$ of kale, arugula and cabbage except mustard, which had a higher stem under B than R of 100 $\mu mol \cdot m^{-2} \cdot s^{-1}$, but shorter under 50 $\mu mol \cdot m^{-2} \cdot s^{-1}$	(Kong et al., 2019a)
Arugula and mustard seedlings	100	24/16	Promoted hypocotyl length compared to R LED, and the promotion effect was greater under 24h photoperiod compared to 16 h	(Kong et al., 2019b)
Arugula and mustard seedlings	20-650	24h	B promoted hypocotyl length compared to R LED from 20 to 650 $\mu mol \cdot m^{-2} \cdot s^{-1}$, although the promotion	(Johnson et al., 2020)

			effect was more obvious for arugula compared to mustard	
Cherry tomato seedlings	205	12	Higher stem length than under monochromatic R, green and white LEDs and fluorescent light	(Kim et al., 2014)
Sesame seedlings (after cotyledon stage)	80 $\mu mol \cdot m^{-2} \cdot s^{-1}$	Not mentioned	Main stem was twice as long as under monochromatic R	(Hata et al., 2013)

The R to FR light ratio is the best characterized shade signal, which decreases in response to canopy density and initiates plant morphological changes to capture more light. This shade avoidance response under changing R and FR is mainly controlled by phytochrome B (Franklin, 2008). Besides, plant hormones are one of the intermediaries that are also involved in stem elongation, among which auxin [indole-3-acetic acid (IAA)] and gibberellin (GA) play the main roles (Yang and Li, 2017). For example, increased level of IAA and GA in the leaves of sunflower (Kurepin et al., 2007a; 2007b), and enhanced biosynthesis of GA in Arabidopsis were found under FR enrichment environment (Hisamatsu et al., 2005; Kurepin et al., 2007b). Evidence has demonstrated that the hormones are involved in shade avoidance response through a partially phytochrome independent signaling (Keller et al., 2011).

Although previous studies showed that plant hormones closely regulate elongation response under shade environment, these hormone syntheses and signaling vary with shade signals. For example, stem elongation of *Arabidopsis* is promoted under a shade signal of B light depletion, and hormone biosynthesis-deficient mutants indicated the biosynthesis and transport of auxin, and GA signaling were not required to induce shade avoidance responses,

but brassinosteroid response was required (Keller et al., 2011). Besides, under FR light enriched environment, the ratio of auxin to GA, rather than their absolute levels, followed the trend of growth and biomass allocation in soybean tissues (Yang et al., 2018). Therefore, it is unknown that hormone signaling circuits triggered by the reduced R:FR ratio and/or B light depletion are also recruited to elicit shade avoidance responses under monochromatic B light.

Light quality also alters the syntheses of secondary metabolites, for example, flavonoids. B light has been found to increase flavonoid biosynthesis, by improving the gene expression of key enzyme, like chalcone synthase (CHS), in its biosynthetic pathway (Liu et al., 2018). For example, in embryogenic calli, the flavonoid content under monochromatic B light was higher compared to under monochromatic R, G or white light at a *PPFD* of 32 $\mu mol \cdot m^{-2} \cdot s^{-1}$ (Li et al., 2018). Besides, B light also increased the total flavonoids at day 20, and flavonol derivatives (i.e., kaempferol and quercetin) at day 40 and 60 of Cyclocarya (*Cyclocarya paliurus*) compared to R light of 800 $\mu mol \cdot m^{-2} \cdot s^{-1}$ (Liu et al., 2018). A significant increase of total flavonoid content was found under B light at 30 $\mu mol \cdot m^{-2} \cdot s^{-1}$ with a short period of 6 d on pea sprout, compared to yellow (Y, 585 nm) and R light (Liu et al., 2016), indicating the potential of B light-induced flavonoid biosynthesis even under low intensity and short growth period. Flavonoids, including flavonols and anthocyanins, are the most widespread and diverse group of polyphenols in *Brassica* species (Cartea et al., 2011). However, there is not enough research that has investigated the content of flavonoids under different light quality at a moderate *PPFD* (i.e. around 100 $\mu mol \cdot m^{-2} \cdot s^{-1}$) during seedling stage in *Brassicaceae* microgreens species. Moreover, the responses could greatly vary with species, owing to the distinct qualitative and quantitative differences in flavonoid profiles within the *Brassica* crops (Cartea et al., 2011).

The alterations of flavonoids under different light qualities further affect the hormone signalling, thereby regulating plant elongation and growth. For example, it is well documented that flavonoids negatively regulate the auxin transportation, as they competing with auxin efflux inhibitor (Besseau et al., 2007; Brown et al., 2001; Taylor and Grotewold, 2005). Flavonoid bisglycosides also affect auxin metabolites and precursors (Kuhn et al., 2016). Interestingly, for the aforementioned experiment on embryogenic calli, the highest flavonoid content was accompanied with the highest growth rate under B light compared to monochromatic R, G or white light under the same *PPFD* (Li et al., 2018). However, this result, as well as the stem elongation effect under monochromatic B reported by many other studies, are somehow contradictory with the current underlined mechanism. Flavonoids should have been increased under B light, and further negatively affect auxin polar transportation and possibly inhibit stem elongation. The contradictory response indicates that other hormones (e.g., GA) are involved in the promotion effect under B light. In a recent study on petunia, B light enhanced the shoot elongation compared to R and RB under a *PPFD* of 70 and 150 $\mu mol \cdot m^{-2} \cdot s^{-1}$, which is accompanied with increases of bioactive GA1 and GA4 contents possibly through the cryptochrome signaling (Fukuda et al., 2016). However, whether the flavonoid profiles in *Brassicaceae* family during the seedling stage of microgreens would affect auxin biosynthesis and transportation, and whether other hormones are involved in regulating stem elongation are still largely unclear.

To explore how light quality from LED light regulates plant development, the objectives of the present study will be (1) to investigate the effect of light quality (i.e., monochromatic B light, R light and RB combination) on stem elongation and its underlying mechanism though the

hormonal regulation; (2) to study the effect of light quality on flavonoid biosynthesis; (3) to investigate if there is any correlation between flavonoid biosynthesis and hormone biosynthesis during the seedling stage of two microgreen species. It is hypothesized that under monochromatic B light, bioactive GAs are promoted, playing a major role in promoting stem elongation of investigated microgreens. B light also promotes the biosynthesis of flavonoid, which may affect the content and signaling of IAA.

Materials and methods

Experimental design and growing environment

The experiment is a 3 × 2 × 2 factorial in a split-split plot design. Two species of microgreens will be grown under three light treatments (B, R and RB-LED) and two levels control of GA biosynthesis (with or without GA-inhibitor treated). The experiment will be conducted in three compartments in a walk-in growth chamber, and the compartments are separated by the opaque white vertical blinds. LED arrays (LX601C, Heliospectra AB, Gothenburg, Sweden) will be used to provide light treatments: (1)B: 100% B (peak at 445 nm); (2)R: 100% R (peak at 660 nm); (3) RB: B and R combination ($B_{90}R_{10}$). All light treatments will be set at the same *PPFD* of 100 $\mu mol \cdot m^{-2} \cdot s^{-1}$with 24-h photoperiod. The temperature and relative humidity (RH) will be set at 20 °C and 70%, respectively, and monitored by Argus system (Argus Control Systems Ltd., Surrey, BC). The experiment will be replicated three times over time. Before commencing each replicate, light intensity and spectra will be measured at 16 spots in the treatment area (50 × 50 cm) to achieve the target *PPFD*, using a radiometrically-calibrated spectrometer (Flame-S, Ocean Optics, Inc., Dunedin, FL) with a 25 µm slit, coupled to

a 1.89 m × 400 µm fiber optic patch chord with a CC-3 cosine corrector. Light intensity and quality will also be checked at the end of each treatment.

Growing media and seeding

Seeds of arugula (*Eruca sativa* L., 'unknown name', Suba Seeds Company S.P.A., Longiano, FC, Italy) and mustard (*Brassica juncea* L. 'Mizuna', Johnny's Selected Seeds, Winslow, ME, USA) will be sowed in pots (8.5cm × 8.5cm × 9cm), which are filled with Sun- shine Mix #5 substrate (Sun Gro Horticulture, MA) and covered with a layer of coconut coir. The seeding rate is 35 $g \cdot m^{-2}$ for both species (i.e., 0.25 g/pot). 10 pots of each species will be put in a supporting tray, and placed under each light treatment. All trays will be top irrigated before germination, and will be sub-irrigation after germination using nutrient solution. A 20–8–20 water soluble fertilizer (Master Plant-Prod Inc., Brampton, ON, Canada) will be dissolved in well water and used for sub-irrigation once a day.

The use of GA-inhibitor

In this experiment, a GA-inhibitor will be used to reduce the level of GA, therefore investigating if GA is involved in B light induced stem elongation effect compared to non GA-inhibitor treated plants. The commonly used GA-inhibitor including Paclobutrazol (PBZ) (Falcioni et al., 2018), uniconazole (Fukuda et al., 2016) and AMO-1618 (Kurepin et al., 2011). PBZ, an ent-kauren oxidase enzyme inhibitor, which efficiently inhibits the biosynthesis of GA will be used in current experiment. The application of PBZ in other studies are summarized in table 2. PBZ is mainly absorbed by roots and stems, and can also be absorbed by leaves, although to a lesser extent (Runkle, 2012). At 5,7 and 9 d after sowing, 100 $mg \cdot L^{-1}$ of PBZ will be applied at the substrate level at the rate of 50 ml/pot in half of the pots (i.e., 5 pots per species), 30 min after

irrigation. For the other half of the pots, plants will receive the same amount of distilled water as control.

Table 2. Examples of research with paclobutrazol application

Species	Stage	Concentration	Application	Results	Reference
Soybean	germination	200 µM paclobutrazol(~48mg·L^{-1})	Petri dishes with 30ml water/water with PBZ	expressed cell wall metabolism genes with PBZ, but promoted photosynthetic genes	(Gazara et al., 2019)
Potato	whole stage	29.9% active ingredient was applied at a rate of 250 g·ha^{-1}	28/35/42 DAP spray	Early applied inhibit stem elongation and sugar content, but increase starch content	(Mabvongwe et al., 2016)
Paddy	field whole stage	100mg-600mg·L^{-1}	55-57 DAS spray	100mg/L not significant 200,400,600mg/L decrease GA3 concentration	(Syahputra et al., 2013)
Rice	Germination and vegetative growth	0, 12.5, 25, 50, 100ppm	Media with seeds were then wetted with PBZ solutions	Height of plants decreased in accordance with increased paclobutrazol concentration	(Dewi et al., 2016)
Camelina	Flowering	25-125mg·L^{-1} 25% active ingredient	At the base of stem 50ml/plant		(Kumar et al., 2012)

Growth and appearance quality measurement

Height of 15 individual plants under each treatment for each species will be measured randomly and recorded daily after seed germination. Plants will be harvested 12 DAS for both

species. For three pots of the microgreens under each treatment, all plants from each pot will be cut from substrate level and weighed for fresh weight (FW). The plants will be collected in paper bags and placed in a drying room at 65 °C, dry weight (DW) will be determined till they reach a constant weight. Another 15 representative plants will be selected from each treatment, and plant height and hypocotyl length will be measured using a ruler. The cotyledons with petioles will be cut from the stems for another 15 plants, and two parts will be scanned using a digital scanner (LiDE 25, Canon Canada Inc., Brampton, ON, Canada). Cotyledon area, cotyledon color, cotyledon width, petioles length, stem diameter will be measure using image J software (1.42, National Institute of Health). After scanning, the two parts will be weighed separately and placed in different bags into the drying oven to determine dry weight (DW). Specific leaf area (SLA) will be calculated. Chlorophyll content index (CCI) of cotyledons under each treatment will be determined with 15 measurements on 15 different plants, using a SPAD meter (SPAD 502; Spectrum Technologies, Inc., Aurora, IL).

Preparation of phytochemical and phytohormones analyses

Under each treatment, 6 subsamples of microgreens with 5 g each will be cut from substrate level, and 3 samples will be put into paper bags and dried till constant weight. The other 3 subsamples will be separated in to two parts, hypocotyl and cotyledon (with petiole), and put in different conical tubes. All tubes will be immediately flash frozen in liquid nitrogen (N_2), stored at -80 °C freezer and freeze dried before analyses. The freeze dried and dried samples will be ground into fine powder in liquid N_2 with sea sands for hormones and flavonoid quantification, respectively.

Determination of flavonoid contents

Flavonoids will be extracted followed the ultrasonic-assisted method developed by (Huang et al., 2009). Briefly, 25 mL of 50% ethanol will be added to each dried sample of around 2 g. The samples will be placed in ultrasonic bath for 60 mins at 55 °C. Samples will be then collected against 0.45 µm microporous membrane. The total and selected flavonoid content will be determined using a colorimetric method and high-performance liquid chromatography (HPLC) system, respectively, as described by Liu et al. (2018). Around 0.5 mL of extraction will be taken, mixed with 0.15 ml of 5% $NaNO_2$ and kept at room temperature for 5 min. Then 0.15 ml of 10% $AlCl_3 \cdot 6H_2O$ will be added and the mixture will be incubated again for 5 min. Afterward, 1ml of NaOH will be added and incubated for another 15 min. The samples will be read using a spectrophotometer with absorbance at 415 nm. The total flavonoid content will be calculated against a standard rutin curve and expressed as mg rutin equivalent/ g DW. Selected flavonoids (i.e. quercetin and kaempferol) will be analyzed using a HPLC system. The extraction will be filtered with filter paper before separating on a C18 column at 25 °C. The mobile phase of the assay includes A (98% acetic acid in water, v/v) and B (100% acetonitrile) with a flow rate of 0.6 $mL \cdot min^{-1}$. The detection wavelength is 360nm. Standards of quercetin and kaempferol will be purchased and a standard curve will be calibrated. The content of selected flavonoids in each sample will be calculated against the standard curve.

Determination of plant hormones

The analysis of GAs and IAA will follow the method of Kurepin et al. (2007a). Briefly, the samples will be extracted at 4 °C in 80% ice-cold methanol (v/v). Deuterium-labeled [$^2H_2$] GAs and [$^{13}C_6$] IAA will be used as internal standards. The ratio of endogenous GAs and internal standards will be kept at around 1:1. Then the extract will be filtered through filter paper, and

purified with a C18 preparative column filled with preparative reserved-phase material. The eluate will be dried at 35 °C and dissolved in 1 ml of 10% aqueous methanol with 1% acetic acid and injected into a reverse phase HPLC. The C18 HPLC fractions that contain IAA and GAs will be dried and methylated, as details described by Kurepin et al. (2006). For GA analysis, the methylated samples will be trimethylsilylated. The identification and determination of IAA and GA will be carried out using a gas chromatograph (GC) connected to a mass spectrometer (MS) in the selected ion monitoring mode. The GC retention time of the endogenous GA and IAA and their isotope-labelled standard will be compared, and the GA and IAA contents will be quantified based on stable isotope dilution equations.

Data analyses

Data will be analyzed by SPSS statistical software (Version 25.0, IBM, New York, USA). Treatment effects will be determined by two-way analysis of variance (ANOVA), and mean separations will be performed using Tukey's honestly significant difference (HSD) test at $P \leq 0.05$. The correlation between flavonoid and auxin contents will be tested using Pearson correlation method.

Summary

During the transition from seed to seedling stage, light and homeostasis of plant hormones are important factors that modulate plant development (Warpeha and Montgomery, 2016). The results of this study will provide profound understanding of light and hormone interaction in regulating plant development and the interaction between hormone and flavonoid metabolism.

Literature cited

Bennett, R.N., E.A.S. Rosa, F.A. Mellon, and P.A. Kroon. 2006. Ontogenic profiling of glucosinolates, flavonoids, and other secondary metabolites in *Eruca sativa* (salad rocket), *Diplotaxis erucoides* (wall rocket), *Diplotaxis tenuifolia* (wild rocket), and *Bunias orientalis* (Turkish rocket). J. Agric. Food Chem. 54:4005–4015.

Besseau, S., L. Hoffmann, P. Geoffroy, C. Lapierre, B. Pollet, and M. Legrand. 2007. Flavonoid accumulation in *Arabidopsis* repressed in lignin synthesis affects auxin transport and plant growth. Plant Cell 19:148–162.

Björkman, M., I. Klingen, A.N.E. Birch, A.M. Bones, T.J.A. Bruce, T.J. Johansen, R. Meadow, J. Mølmann, R. Seljåsen, L.E. Smart, and D. Stewart. 2011. Phytochemicals of Brassicaceae in plant protection and human health – Influences of climate, environment and agronomic practice. Phytochemistry 72:538–556.

Brown, D.E., A.M. Rashotte, A.S. Murphy, J. Normanly, B.W. Tague, W.A. Peer, L. Taiz, G.K. Muday, N.C.D.E. B, S. Cruz, and A.S.M. California. 2001. Flavonoids act as negative regulators of auxin transport in vivo in arabidopsis 1. Plant Physiol. 126:524–535.

Cartea, M.E., M. Francisco, P. Soengas, and P. Velasco. 2011. Phenolic compounds in *Brassica* vegetables. Molecules 16:251–280.

Dewi, K., R.Z. Agustina, and F. Nurmalika. 2016. Effects of blue light and paclobutrazol on seed germination, vegetative growth and yield of black rice (*Oryza Sativa* L. 'Cempo Ireng'). Biotropia. 23:84–95.

Falcioni, R., T. Moriwaki, E. Benedito, C.M. Bonato, L.A. de Souza, and W.C. Antunes. 2018. Increased gibberellin levels enhance light capture efficiency in tobacco plants and promote dry matter accumulation. Theor. Exp. Plant Physiol. 30:235–250.

Fantini, E. and P. Facella. 2020. Cryptochromes in the field: How blue light influences crop development. Physiol. Plant. 0:1–11.

Franklin, K.A. 2008. Shade avoidance. New Phytol. 179:930–944.

Franklin, K.A. and G.C. Whitelam. 2005. Phytochromes and shade-avoidance responses in plants. Ann. Bot. 96:169–175.

Fukuda, N., C. Ajima, T. Yukawa, and J.E. Olsen. 2016. Antagonistic action of blue and red light on shoot elongation in petunia depends on gibberellin, but the effects on flowering are not generally linked to gibberellin. Environ. Exp. Bot. 121:102–111.

Fukuda, N., Y. Ishii, H. Ezura, J.E. Olsen. 2011. Effects of light quality under red and blue light emitting diodes on growth and expression of FBP28 in petunia. Acta Hortic. 907:361–366.

Gazara, R.K., E.A.G. de Oliveira, B.C. Rodrigues, R. Nunes da Fonseca, A.E.A. Oliveira, and T.M. Venancio. 2019. Transcriptional landscape of soybean (*Glycine max*) embryonic axes during germination in the presence of paclobutrazol, a gibberellin biosynthesis inhibitor. Sci. Rep. 9:1–12.

Hata, N., Y. Hayashi, E. Ono, H. Satake, A. Kobayashi, T. Muranaka, and A. Okazawa. 2013. Differences in plant growth and leaf sesamin content of the lignan-rich sesame variety “Gomazou” under continuous light of different wavelengths. Plant Biotechnol. 30:1–8.

Hansen, B. 2000. More over, baby greens. Los Angeles Times. Los Angeles. 26 July 2000. <http:// www. Chicogotribune.com>

Heo, J., C. Lee, D. Chakrabarty, and K. Paek. 2002. Growth responses of marigold and salvia bedding plants as affected by monochromic or mixture radiation provided by a light-emitting diode (LED). Plant Growth Regul. 38:225–230.

Hernández, R. and C. Kubota. 2016. Physiological responses of cucumber seedlings under different blue and red photon flux ratios using LEDs. Environ. Exp. Bot. 121:66–74.

Hisamatsu, T., R.W. King, C.A. Helliwell, and M. Koshioka. 2005. The involvement of gibberellin 20-oxidase genes in phytochrome-regulated petiole elongation of Arabidopsis. Plant Physiol. 138:1106–1116.

Huang, W., A. Xue, H. Niu, Z. Jia, and J. Wang. 2009. Optimised ultrasonic-assisted extraction of flavonoids from *Folium eucommiae* and evaluation of antioxidant activity in multi-test systems in vitro. Food Chem. 114:1147–1154.

Johnson, R.E., Y. Kong, and Y. Zheng. 2020. Elongation growth mediated by blue light varies with light intensities and plant species: A comparison with red light in arugula and mustard seedlings. Environ. Exp. Bot. 169:103898.

Keller, M.M., Y. Jaillais, U. V. Pedmale, J.E. Moreno, J. Chory, and C.L. Ballaré. 2011. Cryptochrome 1 and phytochrome B control shade-avoidance responses in Arabidopsis via partially independent hormonal cascades. Plant J. 67:195–207.

Kim, E.Y., S.A. Park, B.J. Park, Y. Lee, and M.M. Oh. 2014. Growth and antioxidant phenolic compounds in cherry tomato seedlings grown under monochromatic light-emitting diodes. Hortic. Environ. Biotechnol. 55:506–513.

Kong, Y., D. Kamath, and Y. Zheng. 2019a. Blue versus red light can promote elongation growth independent of photoperiod: A study in four Brassica microgreens species. HortScience 54:1955–1961.

Kong, Y., K. Schiestel, and Y. Zheng. 2019b. Pure blue light effects on growth and morphology are slightly changed by adding low-level UVA or far-red light: A comparison with red light in four microgreen species. Environ. Exp. Bot. 157:58–68.

Kong, Y., M. Stasiak, M.A. Dixon, and Y. Zheng. 2018. Blue light associated with low phytochrome activity can promote elongation growth as shade-avoidance response: A comparison with red light in four bedding plant species. Environ. Exp. Bot. 155:345–359.

Kong, Y. and Y. Zheng. 2020. Phototropin is partly involved in blue-light-mediated stem elongation, flower initiation, and leaf expansion: A comparison of phenotypic responses between wild *Arabidopsis* and its phototropin mutants. Environ. Exp. Bot. 171:103967.

Kuhn, B.M., S. Errafi, R. Bucher, P. Dobrev, M. Geisler, L. Bigler, E. Zazímalová, and C. Ringli. 2016. 7-Rhamnosylated Flavonols Modulate Homeostasis of the Plant Hormone Auxin and Affect Plant Development. J. Biol. Chem. 291:5385–5395.

Kumar, S., S. Ghatty, J. Satyanarayana, A. Guha, B.S.K. Chaitanya, and A.R. Reddy. 2012. Paclobutrazol treatment as a potential strategy for higher seed and oil yield in field-grown *Camelina sativa* L. Crantz. BMC Res. Notes 5:137.

Kurepin, L. V., R.J.N. Emery, R.P. Pharis, and D.M. Reid. 2007a. The interaction of light quality and irradiance with gibberellins, cytokinins and auxin in regulating growth of *Helianthus annuus* hypocotyls. Plant, Cell Environ. 30:147–155.

Kurepin, L. V., R.P. Pharis, D.M. Reid, and C.C. Chinnappa. 2006. Involvement of gibberellins in the stem elongation of sun and shade ecotypes of *Stellaria longipes* that is induced by low light irradiance. Plant, Cell Environ. 29:1319–1328.

Kurepin, L. V., S. Shah, and D.M. Reid. 2007b. Light quality regulation of endogenous levels of auxin, abscisic acid and ethylene production in petioles and leaves of wild type and ACC deaminase transgenic *Brassica napus* seedlings. Plant Growth Regul. 52:53–60.

Kurepin, L. V., L.J. Walton, R.P. Pharis, R.J.N. Emery, and D.M. Reid. 2011. Interactions of temperature and light quality on phytohormone-mediated elongation of *Helianthus annuus* hypocotyls. Plant Growth Regul. 64:147–154.

Lee, J.S., C.A. Lee, Y.H. Kim, and S.J. Yun. 2014. Shorter wavelength blue light promotes growth of green perilla (*Perilla frutescens*). Int. J. Agric. Biol. 16:1177–1182.

Li, H., Y. Lin, X. Chen, Y. Bai, C. Wang, X. Xu, Y. Wang, and Z. Lai. 2018. Effects of blue light on flavonoid accumulation linked to the expression of miR393, miR394 and miR395 in longan embryogenic calli. PLoS One:1–22.

Liu, H.K., Y.Y. Chen, T.T. Hu, S.J. Zhang, Y.H. Zhang, T.Y. Zhao, H.E. Yu, and Y.F. Kang. 2016. The influence of light-emitting diodes on the phenolic compounds and antioxidant activities in pea sprouts. J. Funct. Foods 25:459–465.

Liu, Y., S. Fang, W. Yang, X. Shang, and X. Fu. 2018. Light quality affects flavonoid production and related gene expression in *Cyclocarya paliurus*. J. Photochem. Photobiol. B Biol. 179:66–73.

Mabvongwe, O., B.T. Manenji, M. Gwazane, and M. Chandiposha. 2016. The effect of paclobutrazol application time and variety on growth, yield, and quality of potato (*Solanum tuberosum* L.). Adv. Agric. 2016:1–5.

Neff, M.M. and J. Chory. 1998. Genetic interactions between phytochrome A, phytochrome B, and cryptochrome 1 during Arabidopsis development. Plant Physiol. 118:27–36.

Olschowski, S., E.M. Geiger, J. V. Herrmann, G. Sander, and H. Grüneberg. 2016. Effects of red, blue, and white LED irradiation on root and shoot development of *Calibrachoa* cuttings in comparison to high pressure sodium lamps. Acta Hortic. 1134:245–250.

Runkle, E. 2012. Successful use of paclobutrazol. Greenhouse Product News. https://gpnmag.com/article/successful-use-paclobutrazol/

Smith, H. 1982. Light quality, photoperception, and plant strategy. Annu. Rev. plant Physiol. 33:481–518.

Syahputra, B.S.A., U.R. Sinniah, S.O.S. Rastan, and M.R. Ismail. 2013. Changes in gibberellic acid (GA3) content in *Oryza sativa* due to paclobutrazol treatment. J. food pharmaceurical Sci. 1:14–17.

Taiz, L., E. Zeiger, I.M. Møller, and A. Murphy. 2015. Plant physiology and development. Sixth Edition. Sinauer Associates.

Taylor, L.P. and E. Grotewold. 2005. Flavonoids as developmental regulators. Curr. Opin. Plant Biol. 8:317–323.

Treadwell, D.D., R. Hochmuth, L. Landrum, and W. Laughlin. 2016. Microgreens: A new specialty crop. Univ. Florida IFAS Ext. Bul. HS1164.

Wang, H. and X. Deng. 2004. Phytochrome signaling mechanism. Am. Soc. Plant Biol.:1–28.

Warpeha, K.M. and B.L. Montgomery. 2016. Light and hormone interactions in the seed-to-seedling transition. Environ. Exp. Bot. 121:56–65.

Xiao, Z., G.E. Lester, Y. Luo, and Q. Wang. 2012. Assessment of vitamin and carotenoid concentrations of emerging food products: Edible microgreens. J. Agric. Food Chem. 60:7644–7651.

Yang, C. and L. Li. 2017. Hormonal regulation in shade avoidance. Front. Plant Sci. 8:1527.

Yang, F., Y. Fan, X. Wu, Y. Cheng, Q. Liu, L. Feng, J. Chen, Z. Wang, X. Wang, T. Yong, W. Liu, J. Liu, J. Du, K. Shu, and W. Yang. 2018. Auxin-to-gibberellin ratio as a signal for light intensity and quality in regulating soybean growth and matter partitioning. Front. Plant Sci. 9:1–13.